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LIVER DISEASE

Consulting Editor

SHEILA SHERLOCK

M D F.R.C.P

Editor for the Ciba Foundation

G E W WOLSTENHOLME

O B E M A M B B Ch

With 112 Illustrations



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FOREWORD

by

PROFESSOR G R CAMERON, D SC, F R C P F R S

Early in 1950 the Ciba Foundation was asked by Dr Sheila Sherlock whether it would sponsor an international conference on liver disease in the summer of that year The forthcoming physiological conference at Copenhagen meant that research workers from America and Europe would be assembling about that time and, with a little encouragement, they might be persuaded to come to London and join British colleagues in a liver symposium The present volume is the outcome of Dr Sherlock s idea and drive and the Ciba Foundation's initiative, it will be obvious to the reader of the papers and discussions now published how successful was the enterprise and what its bearing on international co operation might well be All such assemblies when well planned seldom fail in their formal purpose, some however achieve more than technical success when a warm, friendly atmosphere envelops the meetings and overflows into the social events and leisure hours offered to the participants That this was the case with the Ciba conference is not surprising since the genial Sec retary to the Executive Council of the Foundation Dr G E W Wolstenholme and his courteous staff devoted them selves to the comfort and indeed the happiness of conference members The scientific results attained were highly satis factory despite the need for restricting discussion to a portion of the vast field of hepatic investigation With commendable foresight Dr Sherlock and her planning committee insisted that this first gathering should concentrate on recent trends in the ætiology of chronic liver disease, the outcome was impressive and few could have foreseen the wealth of dis coveries and ideas which was given to the conference The

Ciba Foundation has made available to all who are interested in the liver these contributions in the form of a permanent record. It will be appreciated that for the most part these do not consist of papers fully prepared for publication but are rather the record of an informal and happy occasion

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CHAIRMAN G R CAMERON, DSC, FRCP, FRS

Part IV—PIGMENT METABOLISM

CHAIRMAN C J WATSON MS MD PHD

List of those participating in or attending the Conference 17th and 18th July, 1950

JOHN BEATTIE	University of Cambridge
C. H BEST	University of Toronto
M BJORNEBOE	Kommunehospitalet Copenhagen
W H BRADLEY	Ministry of Health, London
G R CAMERON	University College Hospital London
P CAZAL	University of Montpellier
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E R CULLINAN	St Bartholomew s Hospital, London
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E J DELORME	University of Edinburgh
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MILES DRAKE	University of Pennsylvania
H DUCCI	Hospital Salvador, Santiago Chile
H FINK	University of Cologne
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H P HIMSWORTH	Medical Research Council London
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ROBERT M KARK	University of Illinois
W A E KARUNARATNE	University of Colombo Ceylon
E J KING	Postgraduate Medical School London
H W KOSTERLITZ	University of Aberdeen

H G KUNKEL	Rockefeller Institute New York *
O LINDAN	University College Hospital London
N F MACLAGAN	Westminster Hospital London
F O MACCALLUM	Central Public Health Laboratories London
T B MAGATH	Mayo Foundation Rochester U S A
J MCMICHAEL	Postgraduate Medical School London
J W MCNEE	University of Glasgow
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J STOKES JR	University of Pennsylvania
G VERDONK	University of Ghent
G VERDONK (MME)	University of Ghent
G VIOLLIER	Medizinische Universitatsklinik Basle
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PART I

PROTEIN METABOLISM IN LIVER DISEASE

Chairman E J. King

RECENT DEVELOPMENTS IN FLOCCULATION TESTS

N F MACLAGAN

The principal development in the field of flocculation tests in the last few years has, I think, been the inception of a new group of tests stemming directly from the work of Cohn and his collaborators, published in 1946, on the refinements of salting out procedure, and particularly interesting are these new tests which measure more exactly gamma-globulin content and which do not depend, therefore, on so many unknown factors Naturally, it would be a great simplification if we could replace these empirical flocculation tests by an actual estimation of some known substance. However, we must not lose sight of the fact that the relationships involved are very complex and if we do, in fact, get extra diagnostic information from a more or less empirical procedure, we must not discard it in favour of these new tests unless it can be proved that the new tests give as much information

I am presenting to-day the results collected in the last year or two at Westminster Hospital, and will try to analyse the value of the new tests from this point of view. Table I shows the tests which are used in the series and the normal limits of each of them

We have had considerable trouble with the question of normal limits, principally on account of the difficulty of

Table I

FLOCCULATION TESTS IN 59 CONTROL SUBJECTS

Test	*Method*	*Normal Limits*
Serum colloidal gold	Maclagan 1944	0
Thymol turbidity	Maclagan 1944	0–2 units
Thymol flocculation	Neefe and Rheinhold 1946	0–1+
$ZnSO_4$ turbidity	Kunkel 1947	0–4 units
$(NH_4)_2SO_4$/NaCl turbidity	de la Huerga and Popper 1949	0–2 units

standardizing turbidity measurements in different laboratories, and the figures given are based on serum protein sulphosalicylic acid standards and not on the barium sulphate standard preferred by Kunkel (1947) Kunkel's test, which was published in 1947, and which we have used a great deal, was standardized on barium sulphate but we have had great difficulty in getting the same results as Kunkel, and we finally went over to the protein sulphosalicylic acid standards You will notice that the thymol turbidity test limits are lower than the ones I had previously suggested I think that is a question of choice of subject, and I must agree with various other writers that if you take entirely normal subjects you do get lower limits than the 0–4 which we originally proposed, and we have gone back to 0–2 for this test The last ammonium sulphate test is one of the new ones One has a choice here between the procedures of Wolfson *et al* (1948) and of de la Huerga and Popper (1950) and our data concerns the latter

Table II will show you some details of the way we standardized our thymol flocculation test

Table II

THYMOL FLOCCULATION TEST

If *incomplete* record as 1+
If completely flocculated record as follows —

Turbidity Units	0–4	5–7	8–10	Over 10
Flocculation	1+	2+	3+	4+

In this test it is always rather difficult to know what to call a 1+ and 2+ and so on, and the methods shown there

do not correspond exactly to the turbidity normals In order to be significant i e more than 1+, the test must show more than 4 units of turbidity and complete flocculation That means that one uses rather stringent criteria for this test which makes it rather insensitive However it has advantages in the study of jaundice

Table III shows the clinical material which we have used

Table III

CLINICAL MATERIAL

Test	*Hepatitis*	*Obstructive Jaundice*	*Miscellaneous*	*Total*
Serum colloidal gold, Thymol turbidity, Thymol flocculation, $ZnSO_4$ turbidity	65	21	88	174
$(NH_4)_2SO_4$/NaCl turbidity	23	4	14	41

It is rather deficient in the latest ammonium sulphate test because this was only published a few months ago The classification here is on a clinical basis and hepatitis includes any sort of generalized liver disease including cirrhosis The miscellaneous group comprises all cases giving positive results which are not included in the other two groups We excluded a good many cases where results were completely negative so as to avoid obscuring the picture There are a number of cases of arthritis in the miscellaneous group because we were especially interested in rheumatism at that time This accounts for the high proportion of positives in the miscellaneous group

Fig 1 shows you the overall results with the different tests The zinc sulphate and thymol give roughly similar overall results Serum colloidal gold and the new ammonium sulphate test also gave about the same percentage of positives in the various groups although you will notice that they are considerably higher than the other two in the miscellaneous group Thus one might say that these tests were less specific Thymol flocculation on the other hand is entirely different

very closely correlated in our results with the zinc sulphate test, and we do not feel it is worthwhile continuing with the gold since the zinc sulphate is easier The ammonium sulphate test, of course, needs to be tried on many more cases It appears to show a smaller range of deviations from the normal as compared with the thymol and zinc sulphate tests It is obviously very tempting, and it would be a great *simplification if we could replace these empirical tests with* a simpler procedure, but I feel that we have not enough data to do so at the present time

Finally I should like to point out the existence of a group of cases of hepatitis, usually cirrhosis, with completely negative flocculation and other liver function tests It would be interesting to know if any of the speakers have any views about the pathology of these cases I think they were described by Sherlock (1946) some years ago as histologically inactive cirrhosis and as asymptomatic portal cirrhosis by Ricketts *et al* (1950) I would like to know if there is anything different in the ætiology or prognosis of this condition My own feeling is that they are usually mild asymptomatic cases but I am not sure that that is always true

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DISCUSSION SERUM CHOLINESTERASE LEVELS IN CIRRHOSIS

ROBERT M KARK

I would like to comment on Dr Maclagan's statement that certain patients with cirrhosis have normal liver function tests Now that is perfectly true After a cirrhotic patient is restored to health, we often find that he shows normal liver function tests Certainly alcoholic cirrhotics who "cure" themselves by going off alcohol and eating an adequate diet will eventually show normal liver function tests

In the past few years, we have been studying the ability of the hepatic cell to manufacture cholinesterase Using the serum cholinesterase as an index of hepatic function, we have found that when other tests are normal, we frequently find a normal *low* serum cholinesterase which, on treatment, goes up to a *high* level, but one which for the patient is still normal The test is a very simple one It was first proposed by Brian McArdle as a test of liver function

To 0 1 ml serum add 10 0 ml veronal buffer at pH 8 0 After adding 1 0 ml acetyl choline bromide, incubate one hour at 25°C During incubation choline and acetic acid are released The acetic acid reduces the pH of the final mixture Readings are made in the Beckmann pH meter and serum cholinesterase is expressed as ΔpH per hour

One patient has been under study for 800 days He started off as a cirrhotic, with a very low serum cholinesterase, and at the 240th day the level was normal At present (800 days) he has gone right up to about 1 5 ΔpH This is quite above normal

So I think that if you have a test that is sensitive enough, and if you follow it in a serial manner on individual patients you might be able to show abnormal values in cirrhotic patients who give normal results by other methods (see Vorhaus Scudamore and Kark, 1950 *Gastroenterology*, 15, 304)

DISCUSSION FACTORS AFFECTING THE RESULTS OF FLOCCULATION TESTS

H POPPER

Dr Maclagan pointed out that from a diagnostic stand point the determination of gamma globulin gives less information than the results of the thymol turbidity or cephalin flocculation or any other of the flocculation tests We have been trying to determine gamma globulin by turbidimetric methods which were checked by electrophoresis (Popper *et al* 1950) We came to the same conclusion especially as far as the differential diagnosis between surgical and medical jaundice is concerned This work clearly indicates that changes in the gamma globulin concentration of the serum are not the main determining factor for the results of the flocculation tests at present used in the differential diagnosis of jaundice We feel that three factors should be listed

The first concerns qualitative changes in the serum albumin as was just clearly shown by Dr Maclagan They cannot be recognized by electrophoretic methods and may be responsible for abnormal flocculation tests in the presence of normal serum albumin and gamma globulin concentrations as determined by electrophoresis Of all the hepatic tests thymol turbidity and cephalin flocculation show the best correlation with histologically recognizable liver cell damage (Popper Steigmann and Szanto 1949)

We consider this change of serum albumin as the most important factor The second factor is a depression of the turbidity or flocculation by regurgitated biliary material It is probably the same factor to which Ducci (1950) has made reference We do not know which biliary substance it is but it could be demonstrated that the zinc sulphate turbidity of Kunkel particularly in contrast to the gamma globulin turbidity is depressed not only by the addition of bile but also by lecithin in concentrations found in serum

We feel that this depressing factor is of great diagnostic importance Because of this factor, the zinc sulphate turbidity has proved one of the most reliable tests to us in the differentiation between surgical and medical jaundice, at least in its early stages A low zinc sulphate turbidity in the presence of abnormal results of other hepatic tests is very strongly suggestive of a surgical type of jaundice (if cholangiolitis can be excluded)

The third factor of importance is change in the gamma globulin concentration as measured, for instance, by turbidity However such changes are helpful in the recognition of chronicity of hepatitis We found that a persistent elevation or even a further rise in gamma globulin while jaundice subsides and the results of the other tests return to normal, is a good diagnostic sign of transition into a chronic stage (possibly into a post necrotic cirrhosis) This elevation is probably related to the activity of the hepatic mesenchyma

The last statement is explained by observation of the basophilic material in the cytoplasm of hepatic epithelial and mesenchymal cells performed by Dr Szanto in our laboratory (Szanto and Popper, in press) This basophilic material, best stained with pyronin, is said to be due to the presence of pentose nucleic acids, which have, in turn been related to protein synthesis (Caspersson, 1950, Brachet, 1950) In the normal liver the epithelial cells are rich in basophilic material, whereas the mesenchymal cells are almost free of it In viral hepatitis and especially in active cirrhosis in which the serum albumin concentration is usually low the epithelial cells are depleted of cytoplasmic basophilic material (The histochemical depletion may, under pathological circumstances not necessarily be borne out by chemical analysis for pentose nucleic acids) In such conditions the Kupffer cells and the histiocytic and other mesenchymal cells in the portal spaces are very rich in basophilia (Fig 1A) The increased amount of pentose nucleic acids in these mesenchymal cells may be taken as a sign of increased protein formation, most probably of the gamma globulins which are known to be

produced by mesenchymal cells Therefore, this mesenchymal reaction (possibly a response to the liver cell damage), is reflected in elevated serum gamma globulin levels In contrast, in obstructive jaundice the Kupffer cells are also proliferated but they are free of cytoplasmic basophilia (and pentose nucleic acids) probably due to the fact that they are loaded with bile pigment (Fig 1B) In keeping with this observation is the fact that in this condition (at least in the absence of a superimposed severe infection), the gamma globulin level is, as a rule, only slightly if at all elevated

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A

B

Fig 1 Photomicrographs of liver sections stained with methyl green pyronin (× 450)

A Portal space in an active Laennec's cirrhosis The accumulated histiocytes have a strongly basophilic cytoplasm which can hardly be differentiated from the nucl

B Carcinoma of the pancreas Bile casts are noted in a dilated bile capillary in left upper corner Two Kupffer cells in the left half are loaded with bile pigment and a large one in the right half contains few pigment granules in a non basophilic cytoplasm

[To face page 10

LYOPHILIC PROPERTIES OF SERA IN LIVER DISEASE

CH WUNDERLY and F WUHRMANN

BETWEEN the blood plasma and tissues like the epithelial layer the reticulo endothelium the liver and the spleen, there is a constant exchange of salts and water, of non electrolytes like carbohydrates and of substances with lipoid solubility (phosphatids steroids, fats) The part which is played by the blood plasma can be compared with the fluid which elutes adsorbed substances in the chromatographic pile Because blood plasma possesses hydrophilic as well as lipophilic properties, very different substances are comprised in this interaction of blood plasma and tissues It is this dual action which makes blood plasma the ideal means for elution and transport As is well known, blood plasma contains the macro molecules of the proteins and therefore forms a com plex colloid system It is characteristic for the latter, that the surface energy is not negligible compared with the volume energy of the system Therefore the enormous surface of the colloid phase is the decisive factor for the transport volume of the blood plasma The surface formation of the plasma proteins as dispersed phase, depends upon their dispersion, a serum with a normal content of albumin has, generally, higher dispersion than a serum where the globulin content is increased In order to evaluate the interdependence of lytic action and protein composition we made use of electrophoresis after the method of Tiselius Philpott Svensson For the measurement of the eluting effect we developed a model adsorption (Wunderly, 1950*a*), it consists of disks of 9 cm diameter which are cut from a thin membrane of animal skin (not Cellophane ') The membranes which we are using are manufactured in Sweden, their trade name is

"Naturin" Such disks of membrane have an approximate weight of 480 mg , they are submerged in 30 cc of Evans blue (2 mg in 100 cc) and others in Sudan red , they are left in the Petri dishes until the colorimetric control shows that each membrane has taken up 400 γ of the dye All the membranes are then thoroughly washed with water, in which the adsorbed dyestuffs are not soluble , next they are placed again in Petri dishes and carefully submerged in dilutions of the sera in saline This dilution is so regulated that the protein content is always 200 mg per 100 cc , hereby a constant quantity of proteins is eluting hydrophilic and fat *dyes under strictly standardized conditions In order to lessen evaporation and contamination, the Petri dishes are covered and placed in the refrigerator They remain there* for forty eight hours after twenty four hours the membranes are turned with a pincette to assure uniform elution After forty eight hours a colorimetric control shows how much of the dye has been eluted The effect is given in γ dye per mg protein The hydrophilic properties of the serum are measured by the elution of Evans blue, an acid Tolidine dye with a molecular weight of 948, and the lipophilic properties by the elution of Sudan red a well known fat dye These measurements in the Pulfrich step photometer from Zeiss are reproducible up to ± 1 2 per cent *The comparison of these data gives a good estimate of the lytic properties of the patient's blood serum* It was interesting to investigate the dependence of the elution effect on the chemical constitution of the dye molecules , to this end we compared the elution of the hydrophilic dyes Benzo blue, acid , Trypan blue acid , Victoria blue, basic , and the fat dyes Sudan black B and Ciba blue B Z L (Wunderly, 1950*b*) These experiments showed us that Evans blue and Sudan red are best qualified for measurement , therefore we use these indicator dyes for routine work The standard elution, as we have outlined it above can also be carried out when serum is *replaced by uro protein like Bence Jones protein or with bile* (Wunderly, 1950*e*) *The results of such* experiments are given in Fig 1

Here we find the elution effect by normal blood serum and by Bence Jones protein, isolated from the urine of a patient with myeloma The latter uroprotein was carefully dialysed and its concentration brought up by pressure dialysis to be equal with normal serum protein

In the elution experiments with bile, the same quantity of

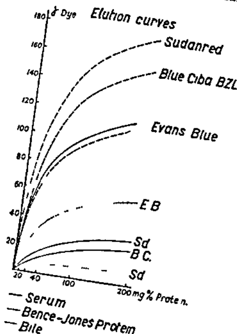


FIG 1

normal bile was used as blood serum The concentrations are given in mg in 100 cc on the abscissa of Fig 1 the eluted dyes in γ are given on the ordinate A comparison of the data given in Fig 1 shows that the lytic action of Bence Jones protein is markedly smaller than of normal serum

protein. Bile shows a strong lipophilic action which is very characteristic; bile elutes even more of the fat dyes, Sudan-red and Ciba blue BZL, than Evans-blue, which effect is just the opposite from the lytic action of the serum proteins. These findings are in line with the function of bile in the metabolism of fats and lipids.

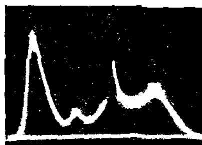
Our *experiments show that the lytic properties of blood* serum depend on albumin and the α- and β globulins. Both these globulins have long been recognized as lipoid carriers and we have been able to prove that their elution capacity depends on their concentration of lipoproteins. The lipotropic action of the latter can be demonstrated if the serum of a patient suffering from nephrosis, for instance, is treated in such a manner as to reduce the lipoproteins and lipids; the subsequent elution of the fat dye Sudan-red was found to decrease by 38 *per cent* and the Evans-blue by 19 *per cent* (Wunderly, 1950*c*, 1950*d*). The binding of Sudan red by the lipotropic portions of the serum proteins is strong enough to remain through dialysis and electrophoresis; we were therefore able to reproduce the red coloured protein boundaries (descending) in the electrophoretic pattern on colour film (Agfacolor). Mobilities of protein boundaries before and after elution of indicator-dyes were not measurably altered. We are presently trying to improve electrophoretic patterns of sera with much bile pigment by preliminary elution of different fat dyes.

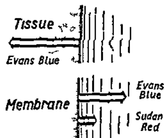
In order to characterize the interaction between the blood stream and surrounding tissues it was necessary to obtain also values measured *in vivo*. We have therefore determined *the disappearance rate of Evans blue* (T-1824) *from* the patient's blood within one hour. Noble and Gregersen (1946) found a mean rate of 5.2 per cent, after measurements on 71 normal individuals. We determine the plasma-volume and, with the hæmatocrit, also the blood volume. The disappearance rate does not only depend on a tissue factor but also on the strength of the binding of the indicator dye to the serum proteins. We measure *this binding capacity of*

a given volume of serum for Evans blue by submerging an undyed membrane in a solution of diluted serum and a constant amount of Evans blue (Wunderly, 1950*a*) In preliminary experiments we have determined the volume of normal blood serum which is capable of binding *all* the Evans blue In such cases no colouring of the membrane takes place, whereas in cases of sera with reduced binding capacity the free dye colours the membrane In order to find out we leave the submerged membrane (see above) for forty eight hours at 2°C After this lapse of time a colorimetric control shows whether the Evans blue content is reduced or not Thus we gain a measure for the binding capacity If we compare it with the disappearance rate of Evans blue *in vivo* we can deduce indirectly the size of the cellular factor It remains a matter of debate which way the free Evans blue dye takes when it leaves the blood stream It is generally agreed that the colloid dispersion of this indicator dye hinders the penetration of the capillary wall, it is therefore well possible that the main process is a pure adsorption on the epithelial layer but it is probable too that the sorptive qualities of the adsorbent like its surface development and composition are modified through disease There is possibly a change from hydrophobic to hydrophilic properties, a change for which we have still no certain means of control The increase of permeability in inflammation is perhaps a parallel (cf Menkin 1940) But illness will not only bring changes of dye concentration at the interface but also alters the degree of dispersion of the colloid indicator dye The average diameter of particles composing the dispersed phase depends on electrolytes and the highly dispersed serum proteins will tend to change the colloidal state of the Evans blue dye sol into a molecular disperse solution a process which is termed dissolution We do not know either whether the serum proteins as the dispersed phase are adsorbed at the interface if parts of the epithelial layer are proteophil then lyosorption would take place and change conditions of membrane equilibrium Such superimposed processes cause local

in the metabolism of proteins and fats The metabolism of bile pigments is only slightly affected Cardiac condition suggestive of so called myocardosis (Wuhrmann)

The protein content of the serum as well as the affinity of serum for Evans blue were normal (Fig 3) On the other hand the dis appearance rate of 14 per cent in the first hour after intravenous injection of Evans blue was greatly increased (normal 5–6 per cent) These figures show that the affinity of the capillary wall for the dye





Alb	α	β	γ	
35	9	28	28	rel %

Total Serumprote n 6 8 per cent
Blood Volume 5 380 ml
Plasma Volume 3 500 ml
78 ml/kg (45 55)
Serum Bilirubin 0 8 mg per cent (—1 3)
Cholesterol total 186 mg per cent {54 free, 48 ester}

FIG 3

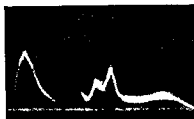
must be particularly great The hydrophilic properties of the serum are normal whilst the increased content of β globulin has lipotropic properties

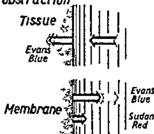
CASE No 2

Male aged 57 Jaundice in extra hepatic biliary obstruction (Cancer of stomach)

for carcinoma of stomach
ee from complaints until
'atient admitted because
Marked hard resistance
of the spleen *no ascites*
myocardos s

For blood chemistry please see Table I
Comment 57 year old male with typical obstructional jaundice





Total Serumprotein 5 6 per cent
Blood Volume 7 870 ml
Plasma Volume 4 410 ml
83 ml/Kg (45 55)
Serum Bilirubin 20 8 mg per cent
Cholesterol total 146 mg per cent {108 free, 26 ester}

FIG 4

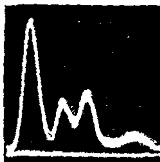
increased

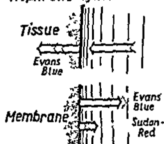
CASE No 3

Male aged 28 Chronic glomerulo-nephritis with nephrotic strain
One year before acute glomerulo nephritis following on tonsillitis not completely cleared up combined with development of a mild

nephrotic syndrome

Comment · Typical nephrotic syndrome in young man after acute glomerulo nephritis Disturbances of liver function were demonstrated





Alb	α	β	γ
46	18	21	15 rel. %

Total Serumprotein 5 3 per cent.
Blood Volume 5,930 ml
Plasma Volume 3,200 ml
53 ml/Kg (45-55)
Serum Bilirubin 0 2 mg per cent
Cholesterol total 289 mg per cent {106 free. 49 ester.

FIG 5

only indirectly by the dysproteinæmia The protein content of the

increased

Summary

It is shown by model adsorption with standardized membranes of animal skin how the elution of hydrophilic Evans blue and lipophilic Sudan red dyes by blood serum, Bence Jones uroprotein and bile can be measured. Thus the lytic properties of the said fluids are compared.

The binding capacity of the patient's blood for Evans blue as well as the disappearance rate of this dye within one hour, serve to characterize conditions *in vivo*.

The sum of the findings form a new reaction combination. Results are shown and explained on cases of hæmochromatosis, jaundice in extra hepatic biliary obstruction (cancer of the stomach) and nephrotic syndrome.

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AMINO-ACID METABOLISM IN LIVER DISEASE

C E DENT and J M WALSHE

It has been known for a long time that there are disturbances of amino acid metabolism in severe liver disease This was originally noted as the result of the identification of crystals of leucine and tyrosine in the urinary deposit obtained from some cases of acute yellow atrophy Later, chemical methods for determining α amino nitrogen were developed and applied to the investigation of such cases Results obtained by this technique confirmed the elevation of the urinary amino acid levels, and showed that the plasma level too was greatly raised

It has been shown in animals that a significant rise of blood amino acids does not occur until about 90 per cent of the liver has been removed By analogy with such experiments, therefore, one would not expect to find, in man, a marked rise in the blood amino acid level until very severe liver damage had occurred Preliminary clinical and experimental work using paper chromatographic techniques (Dent, 1947) tended to confirm this and had led to the conclusion that the test for gross amino aciduria using strips of filter paper (one way chromatography), although of possible value in immediate prognosis of severe liver damage, was useless as a function test in mild cases A conspicuous change in urine amino acids for instance was often absent in cirrhosis and hepatitis

In the work to be described now the problem has been re examined We decided to look not only for the gross changes in output of many amino acids which is so characteristic of the severe forms of liver damage, but also for minor changes of more subtle nature which might involve only one

or two amino acids It seemed reasonable to suppose that the metabolism of some key amino acids might be disturbed long before the liver damage was severe enough to produce the more spectacular gross amino aciduria To detect such minor changes we used only the two way method using squares of filter paper In addition we decided to follow the patients for as long a period as possible, in case some of the changes were only fleeting in character We decided to restrict this preliminary exploration mainly to urine, working on the assumption that if a rise occurs in urinary output this almost certainly reflects a raised plasma level

The cases studied (numbers in brackets) have been of acute yellow atrophy (8), infectious hepatitis (11), fatal liver coma in chronic liver disease (5) cirrhosis (10), obstructive jaundice (3), primary hepatoma (1) i e 38 cases in all In addition for reasons to be explained later we studied 17 cases of patients immediately after major operations under general anæsthesia We have averaged about three analyses of urine at different times from each patient, naturally taking many more specimens from those showing more marked changes

The results have been of some interest We found definite changes in amino acid concentrations in most of the cases The changes were not random in nature involving chance permutations and combinations of amino acid patterns On the contrary they tended to group themselves into about six types of response If, to avoid confusion at this stage by chemical minutiæ, we describe the responses by the letters A–Γ (see Table I) then we will be able to indicate straight away the general nature of our findings These are summarized in Table II, and it can be seen that there is no accurate relation at first sight between the type of amino acid excretion and the diagnosis The only nearly consistent finding was the response A in seven out of eight cases of acute yellow atrophy The response F only occurred in our one case of primary hepatoma It cannot be evaluated as a diagnostic sign till we have more cases of this type That some of the responses are not even specific to liver disease can be seen from the fact that they

may occur in our "post operative" group, some of which have no clinical evidence of liver damage in the ordinary sense of the word

Table I

TYPES OF URINARY AMINO ACID CHANGES FOUND

TYPE A	[illegible]
B	[illegible]
C	[illegible]
D	[illegible]
E	[illegible] excess
F	Ethanolamine alone excreted in excess

Table II

TYPE OF URINARY AMINO ACID RESPONSE

	A	*B*	*C*	*D*	*E*	*F*	*Misfit*
Acute yellow atrophy (8 cases)	7			1			
Fatal liver coma (5 cases)	3	2					
Infectious hepatitis (11 cases)		4	3	2	1		1
Cirrhosis non terminal (10 cases)		1	3	3	1		1 (B+F)
Obstructive jaundice (3 cases)			1				2 normal
Primary hepatoma (1 case)						1	
Post operatives (17 cases)		6	7	4			

Now for the details of the responses They are summarized in Table I Type A is the gross amino aciduria that occurs in acute yellow atrophy We knew this before The one case of the eight which did not show this response was less acute than the others since she died after one month's jaundice We have other evidence also to suggest that the full response only occurs in the really fulminating type B, a milder amino aciduria than A, may also be designated as an 'acute yellow atrophy in miniature' It was a common response in infectious hepatitis especially in the more acute cases with severer and shorter lived symptoms C–F are we believe the responses which are original and unexpected The first three (C–E) involve increased urinary excretions of cystine, with or without 'T spot" (an unidentified new amino acid*) and methyl histidine Generally speaking symptoms were severer in cases showing D and E than those

*Since this paper was read "T-spot has been identified as β amino isobutyric acid (Crumpler Dent Harris and Westall 1951 *Nature* 167 307

with C alone The freak response F we will return to in a moment We must stress that we have over simplified the problem in attempting to put it to you in manageable form We have strong suspicions that there are at least two more groups to be considered one involving aromatic amino acids plus the E changes and another curious one with methionine plus C response We are fully convinced nevertheless that there is some orderly arrangement to be found of the general nature we have described

So far we have not introduced any time scale into the discussion Roughly it seems to be as follows In acute liver damage the urinary response is acute In chronic it is chronic In an acute exacerbation of chronic an additional acute response may also occur We illustrate in Table III the response in successive urines from a case of acute hepatitis in a girl of 13 The strengths of urinary amino acid outputs are expressed in arbitrary units At first a weak D response was exhibited At the height of clinical symptoms (6/4/50) a B response was superimposed This tailed off to practical normality by 16/4/50 when the symptoms had disappeared There is a complication here in that the T spot output was still raised at the end of the jaundice This we believe to be unrelated to her liver disease A small proportion of normal people put out a larger than usual quantity of this substance and we believe this patient s normal was probably 4 and not 1 of our arbitrary units This condition is familial so that being unable to collect a further urine some time later from the patient we obtained some from her sister This also contained a larger concentration of T spot in confirmation of our theory

Table III

A TYPICAL SERIES OF URINARY CHANGES FOUND IN AN EARLY CASE OF INFECTIOUS HEPATITIS

	Normal output	3/4/50	6/4/50	8/4/50	10/4/50	12/4/50	14/4/50	16/4/50	(Normal sister of Patient)
Cystine	<1	3	1	2	2	2	1	<1	—
T spot	1	8	8	7	9	8	3	4	4
Methylhistidine	<1	—	-	1	4	2	—	—	—
All others		+	++	—	+	—	—	—	—

The question as to the specificity of the urinary amino acid responses arose during investigation We began to be sus picious since some of our liver patients showed additional changes after operative procedures such as laparotomy We therefore collected urines before and after various major operations We found that all showed responses similar to those of our liver patients, usually reaching a peak about three days after the operation This certainly requires some inter pretation and further study We suggest that it probably indicates a true liver dysfunction but we cannot yet say if it is a direct effect of the anæsthetic on the liver, or if it is more in the nature of a stress reaction, as a part of the negative nitrogen balance which has long been known to occur

It is clear that we have little here to help us in the diagnosis of these milder types of liver damage Further follow up studies will indicate whether there may yet be something of prognostic value Some recent results suggest that this may be so On the biochemical level however we are at once challenged for an explanation for the special relation that cystine, ' T spot' and methyl histidine bear to the matter of liver dysfunction We are hampered by not yet having a final identification of ' T spot " We should have this soon but all we know so far is that it is a simple aliphatic "non α" amino acid We are fairly sure that this also means that it cannot be a protein building block since such a queer molecule has never yet been found in protein hydrolysates Methyl histidine likewise, does not occur in proteins and has only been described before in the form of the dipeptide anserine which is widely distributed in animal tissue extracts Cystine whilst it can be incorporated into proteins, occurs also in the tripeptide glutathione, indeed most of the non protein cystine of tissue extracts is in this bound form

Our tentative hypothesis is therefore as follows In mild liver disease a few amino acids, normally held in the intra cellular water as peptides, are liberated in free form into the blood stream Of these few only three, cystine, "T spot ' and methyl histidine are slowly metabolized and can therefore

build up appreciable concentrations in the plasma and hence be passed into the urine This theory is merely an extension of long known facts, namely, that when severe liver disease is present the tissue autolytic processes are much more considerable Whole proteins are then broken down and all the common amino acids are rapidly liberated into blood and urine, thus giving us our acute yellow atrophy response If our theory is correct, the kidney plays no part in the causation of the urinary changes and we would anticipate raised plasma levels to occur for each substance found in excessive quantity in the urine So far we have only examined the plasma in six cases of C (cystine alone) response All had greatly increased cystine concentrations but were otherwise fairly normal

A digression here on cystinuria In the usual condition described by this name large excretions of lysine and arginine, as well as of cystine, occur in the urine (Dent and Rose, 1949) The plasma levels of these amino acids are however normal and there must therefore be a "low renal threshold" There are plausible reasons based on chemical structural relations why these three amino acids are linked together in this way It is intriguing to note that in the hepatic cystinuria" which we are describing here, the cystine occurs in the urine without any lysine or arginine Apart from the theoretical importance of this there is also the fact that this enables us to distinguish the two conditions readily on paper chromatograms. We are not surprised that the condition seems to have escaped discovery in the past since in the 'hepatic cystinurias" of mild liver disease (C response) the urinary cystine concentration is less than is found in the 'renal cystinurias," so spontaneous crystallization of the cystine from the stood urine is unlikely —indeed we have so far failed to observe it or to bring this about Another reason is that the cyanide/nitroprusside reaction for cystine (Brand Harris and Biloon 1930) is usually negative in hepatic cystinuria however much cystine may be present We are puzzled as to the reason for this A few drops of urine from a hepatic cystinuria if added

to 2–3 cc of urine from a "renal cystinuria" will suffice to make the cyanide/nitroprusside test negative, and if the drops are added to a test with pure cystine in which the colour is already developed the strong red is rapidly decolourized Another reason why the condition does not make itself conspicuous is that stone formation is not to be expected in hepatic cystinurias owing to the lower cystine concentration In a large series of cases of "renal cystinuria" collected by Lewis (1932) and by Dent and Harris (unpublished), cystine crystallization or stone formation only occurred in the urinary tract of those people with the highest cystine concentrations and then only in a small proportion of them

The last response I concerned a large excretion of ethanolamine in the urine in the absence of any other conspicuous changes detectable on the chromatograms We have often found smaller quantities before in acute yellow atrophy urine when very many other amino acids were present, but never before as an isolated occurrence We took the opportunity to confirm its identity unequivocally by very specific chromatographic methods and finally by actual isolation (Dent, Fowler and Walshe, 1951) The patient was a woman of 45 who had had a swelling, at first painless, of her liver for three years Recently it had become painful and its enormous size caused considerable embarrassment Nevertheless she kept going as a housewife and was in reasonably good general health A biopsy two and a half years ago showed primary hepatoma All the usual liver function tests were normal when carried out on several occasions during the last two years

The known metabolism of ethanolamine is summarized in Fig 1 Its close relation to choline poses interesting biochemical problems Suppose she had for some reason a metabolic arrest at the stage between ethanolamine and its mono methyl derivative? This would produce an accumulation of ethanolamine in the body fluids, including urine, and also the signs of choline deficiency, i e fatty liver→cirrhosis→primary hepatoma (Copeland and Salmon, 1946), The arrest

could theoretically be either due to inability of the liver cells to concentrate ethanolamine, to lack of labile methyl groups, or to absence of sufficient enzyme to carry through

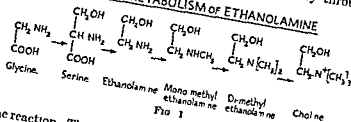


Fig 1

the reaction The other possibility is that there is no metabolic arrest, but on the contrary an overproduction of ethanolamine owing to an uncontrolled synthetic activity of the large mass of tumour cells However we may try and explain it, we feel

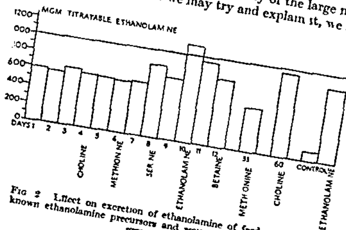


Fig 2 Effect on excretion of ethanolamine of feeding with known ethanolamine precursors and sources of labile methyl groups.

sure the curious metabolic finding is closely linked to the activity of this particular type of tumour tissue and is not a non specific sign of liver dysfunction Fig 2 shows the result

of a series of feeding experiments in which known ethanolamine precursors and sources of labile methyl groups have been fed to the patient It can be seen that feeding methyl donors did not lower the ethanolamine excretion and the patient's response to fed ethanolamine was to excrete less additional ethanolamine than a normal control similarly fed As we are unable to offer a satisfactory explanation of these results we are proposing to carry out isotope experiments with labelled precursors In the meantime we can only add that long term administration of choline has produced no bio chemical change, but has been coincident with considerable relief of pain

Summary

The amino acid excretion in the urine has been studied in both acute and chronic liver disease Certain characteristic changes are described In particular, we have consistently observed a rise in urine cystine levels, often as an isolated finding We suggest that this may be a very sensitive indication of liver damage

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GENERAL DISCUSSION

P GYORGY In connection with Dr Dent's findings, I would like ... ACTH

C E DENT I am afraid I cannot give a ... hereditary cystinurics of course, both Dr Gyorgy and I agree that there

is a low renal threshold for cystine He examined some cases of 'hepatic cystinuria' and found greatly increased blood levels of cystine but we will not be completely satisfied till we have studied more cases As for the changes found in our post-operative group, I still believe that these are due to liver damage but not to what we normally call liver damage in a clinical sense That is why I used the description "stress reaction" I think the output of amino acids in the urine is more likely to be due to a raised blood level In acute yellow atrophy there is a gross rise of the amino acid concentrations in the blood

P GYORGY I am still not convinced that your post operative stress is hepatic rather than renal and I think the adrenal cortex should be considered

C H BEST I take it that Dr Dent estimated choline excretion at the same time as that of amino ethyl alcohol? I don't know whether choline shows up on those paper chromatograms or not

C E DENT We have not yet estimated choline excretion Unfortunately choline does not show up on the chromatograms as we do them

C H BEST It would be interesting to know the choline excretion since so many of blood phospholipids are choline-containing

T C CHALMERS In regard to Dr Dent's observation of an increased excretion of the middle amino acid groups B C, D, E, following major operations we noticed last year, while studying liver function in gastrointestinal hæmorrhage, that the bromsulphalein test stayed fairly normal in spite of frequent shock and severe hæmorrhage, but after operative intervention it became markedly abnormal A change in liver function tests following operations has been noted by others It was also noted by Zamcheck (1949) that liver biopsies taken at operation showed fairly marked acute inflammation of a sort not described in the past, consisting of quite striking centrilobular polymorphonuclear infiltration occasional necrosis of liver cells and subcapsular hæmorrhagic infarcts Biopsies taken before the operation had extended very far as soon as the surgeon got into the abdomen showed none of these acute changes, but when the biopsies were repeated three to five hours later they revealed this fairly marked hepatitis Thus these post operative changes in bromsulphalein retention, aminoaciduria and pigment metabolism might be explained on the basis of focal or diffuse hepatitis associated with the stress of operation It might be due to anæsthesia though we could not correlate it with the type of anæsthesia used or to anoxia or actual local trauma to the liver, since most of these patients were undergoing upper abdominal operations with surgeons pulling very hard on the retractors, and the most marked changes were found in the lobes retracted most.

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CHANGES IN THE CONSTITUENTS OF THE LIVER CELL IN EARLY CHOLINE AND PROTEIN DEFICIENCIES

H W KOSTERLITZ

THIS investigation, which was carried out in collaboration with Miss R M Campbell in the Physiological Laboratory of the University of Aberdeen, was undertaken with a view to elucidating the changes in the essential constituents of the liver cell in early protein and choline deficiencies It appeared desirable to examine the livers when the animals were in a state of equilibrium For this reason, adult (male) rats were used in the post absorptive state, i e twenty two to twenty four hours after the last food had been offered at 4 p m on the previous day In the experiments, in which ^{32}P was used, any food left over at 9 a m was removed each day The diets were fed for one to four weeks The management of the rats the composition of the basal diet and the analytical techniques have been described elsewhere (Campbell and Kosterlitz, 1949 1950)

Deoxyribonucleic acid P (DNA P) was used as a standard to which all other analytical values were referred So far as is known, DNA P is the only constituent of liver tissue which is not affected by variations of the diet (Campbell and Kosterlitz, 1950) It was first shown by Vendrely and Vendrely (1948) and confirmed by Davidson and McIndoe (1949) and Mirsky and Ris (1949) that the DNA P content of a somatic nucleus is approximately constant for any one species and that therefore the DNA P content of a tissue is a good indicator for the number of nuclei present Thus it was possible to establish a quantitative relationship between body weight and liver DNA P (Campbell and Kosterlitz, 1950) This relationship has now been examined for a further 138 rats,

the regression equation calculated from these results is not significantly different from that obtained previously. By analysis of covariance and recalculation of the values for the mean body weight dietary variations of protein fat and choline have been shown to have no influence on the DNA P content of the liver (Fig 1). Similarly fasting up to forty eight hours is without influence which confirms earlier findings on growing rats (Davidson 1947). The only exceptions were

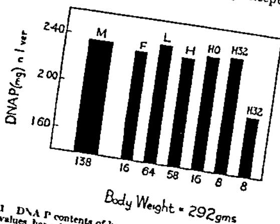


FIG 1 DNA P contents of livers of rats fed on different diets. The values have been adjusted to the mean body weight of 292 g. M mean. F fasted 24–48 hr. L, 10 per cent fat diets. H 40 per cent fat diets. HO protein free 40 per cent fat diets. H32 82 per cent casein 40 per cent fat diet for one and four weeks respectively. The figures below the columns indicate the number of rats in each group.

the rats fed on the diet containing 82 per cent casein and 40 per cent fat for four weeks. In these animals the DNA P content of the liver was smaller than that calculated from the regression equation. However as these animals gained weight excessively during the experimental period this discrepancy is probably only apparent the true body weight being lower than the measured one

It was shown previously (Campbell and Kosterlitz, 1950) that the protein content of each liver cell expressed as the ratio protein N/DNA P, is a function of the dietary protein intake The relationship was found to be linear for casein intakes between 0 and 150–200 *mg casein N/100 g* body

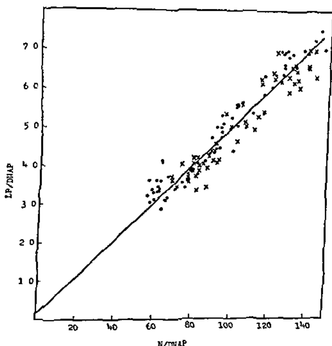


FIG 2 The lipoid P contents of liver cells for different protein N contents

weight The phospholipin content of the liver measured as lipoid P (LP) varies directly with the protein N content and is thus also a function of dietary protein If the ratio LP/DNA P is plotted against protein N/DNA P, a straight line is obtained the origin of which passes through zero (Fig 2) Analysis of covariance and recalculation of the LP/DNA P

ratios for a mean N/DNA P ratio of 97 indicate that choline deficiency does not affect this relationship, while there is a significant difference between low (10 per cent) and high (40 per cent) fat diets, the latter yielding for identical N/DNA P values, a greater LP/DNA P ratio than the former (Fig 8) This means that adult rats with a choline deficiency

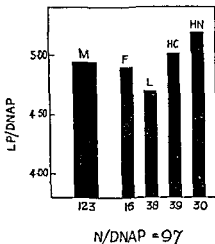


Fig 3 Lipoid P contents of livers of rats fed on different diets

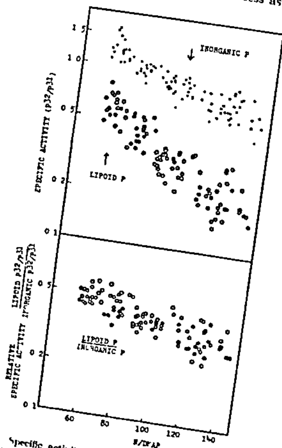
of up to four weeks, when examined in the post absorptive state, show no loss of phospholipins other than that produced by variations in dietary protein, on the other hand, a high fat content of the diet, with or without choline, causes a small increase in the phospholipin content of the liver, in addition to any variations produced by the protein content of the diet

As in the case of the phospholipins, the ribonucleic acid (RNA) content of the adult rat's liver is a linear function of the protein content of the liver, the gains and losses of RNA P are, however, smaller than those of protein N (Campbell and Kosterlitz, 1950) These findings have been confirmed, although it would appear from the analysis of the present series of experiments that factors other than the protein content may play a role For instance, rats fed on a high fat diet containing no choline have a slightly but significantly higher RNA P/DNA P ratio than rats fed on a high fat diet containing choline Rats fasted for twenty four hours show a normal ratio, while it is lowered in animals fasted for forty eight hours

It would appear then that as far as the protein, phospholipin and ribonucleic contents of the liver are concerned, changes in the protein content of the diet have the most important effect, in addition the phospholipin content is slightly affected by variations in dietary fat The effects of choline deficiency are almost negligible, particularly striking is the absence of any effect on the phospholipin content

The second part of this paper deals with the problem of how far deficiency of protein and choline affects the turnover rate of phospholipins in the liver For this purpose, the incorporation of ^{32}P into the phospholipin molecule was determined six hours after the injection of $NaH^{32}PO_4$ Simultaneously with the injection of ^{32}P, any food left over was removed It was found that the specific activity of the lipoid P, viz ^{32}P in per cent of injected counts/^{31}P in mg, rose with decreasing protein and therefore decreasing phospholipin content of the liver At the same time, however, there was a similar change in the specific activity of inorganic P, a fact which in itself would cause a rise of the specific activity of lipoid P In Fig 4 the logarithms of the specific activities of inorganic P and of lipoid P are plotted against the ratios N/DNA P The slope for lipoid P is significantly greater than that for inorganic P, so that the slope of the logarithms of the ratio specific activity of lipoid

P/specific activity of inorganic P (=relative activity of lipoid P) plotted against N/DNA P differs significantly from zero Thus, it would appear that the time taken to renew the lipoid P present in the liver becomes less as the animal



o 4 Specific activities of inorganic P and lipoid P and ative specific activities of lipoid P in rats livers with different protein contents

loses lipoid P from its liver as a consequence of dietary protein deficiency In order to obtain some idea of whether or not the turnover time is sufficiently shortened to compensate for the loss of lipoid P, a quantitative estimation of the turnover time and turnover rate became necessary

Turnover time is the time taken for the whole phospholipin present in the liver to be renewed and turnover rate is the amount of phospholipin renewed per unit of time According to Zilversmit, Entenman and Fishler (1943), the turnover time of lipoid P in hours is equal to the difference between the mean specific activities of the immediate precursor of lipoid P and of lipoid P divided by the increase in specific activity of lipoid P per hour The immediate precursor is likely to be glycerophosphate (Zilversmit, Entenman and Chaikoff, 1948, Popjak and Muir, 1950) In the present experiments use was made of the fact that the P which is resistant to alkaline hydrolysis, alkali stable P, is made up mainly of glycero phosphate P (Zilversmit *et al*, 1948)

In Fig 5, the specific activities of inorganic P, alkali stable P and lipoid P in the liver are plotted for rats which had been fed on a protein free diet for one week The turnover time was determined by the method of Zilversmit *et al* (1943) and was 7 5 hours when calculated for the interval one and a half to three hours, and 7 6 hours for the interval three to six hours *The results obtained for rats fed on a 40 per cent* casein diet for one week are plotted in Fig 6 The turnover times were 10 6 and 9 9 hours respectively

To arrive at the values for turnover rates, the amounts of lipoid P present in the liver were divided by the turnover times As can be seen from Table I, the turnover rate of lipoid P was slightly lower in animals fed on a protein free diet than in those fed on a 40 per cent casein diet It is impossible to say whether this difference is statistically significant or not These results indicate that the reduced amounts of phospholipins which are present in the livers of rats fed on a protein free diet are resynthesized more rapidly and thus more often than in an animal fed on a diet adequate in protein

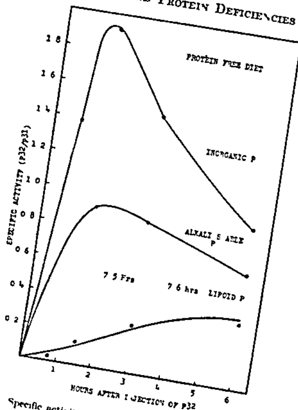


Fig 5 Specific activity—time curves obtained from rats fed on a protein free diet for one week

Table I

Diet	Turnover time hr	Lipoid P mg /300 g body wt	Turnover rate mg /300 g body wt hr
40 per cent casein	10 3	14 4	1 4
Protein free	7 5	8 7	1 15

Apart from the protein content of the diet no other factor was found which affected the turnover of lipoid P in the liver This result was obtained by calculating the regressions of the

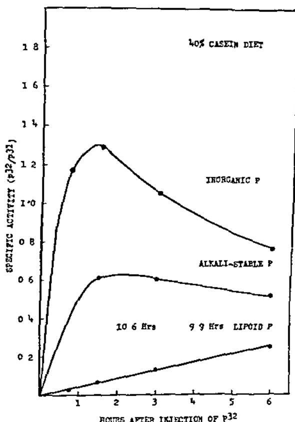


FIG 6 Specific activity—time curves obtained from rats fed on a 40 per cent casein diet for one week.

specific activities of inorganic P and lipoid P on body weight and the ratio N/DNA P By analysis of covariance and calculating the specific activities for the means of the body weights and the N/DNA P ratios no significant differences

were found between rats subjected to different dietary treatments, viz. fasting up to forty-eight hours, low fat diets and high fat diets with and without choline (Fig. 7) This is the more remarkable in that the rats which had been fed on the

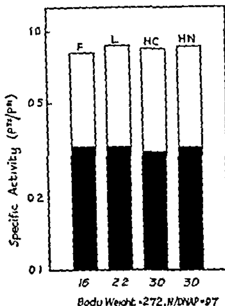


FIG 7 Specific activities of inorganic P (clear columns) and lipoid P (shaded columns) of rats fed on different diets The values have been adjusted to the mean body weight of 272 g and mean N DNA P ratio of 97 F, fasted for 24–48 hr, L, 10 per cent fat diet HC, 40 per cent fat diet with choline, HN, 40 per cent fat diet without choline The figures below the columns indicate the number of rats in each group

diet containing 8 per cent casein and 40 per cent fat without choline for four weeks showed a very considerable fatty infiltration of the liver, 1 86 g triglycerides/mg. DNA P, while their litter mates receiving the same diet plus choline

had only slightly raised triglyceride levels, 0 28 g /mg. DNA P, in their livers

Summarizing, we may say that in the adult rat examined in the post absorptive state, the phospholipin content of the liver and its turnover are determined mainly by the protein content of the diet The fat content of the diet has a slight effect on the phospholipin content but none on the turnover The presence or absence of choline has no effect on the amount of liver phospholipin or its turnover although large amounts of triglycerides accumulate in the liver in choline deficiency

Two questions remain which cannot be answered at present The first concerns the stimulus which increases the turnover of phospholipins in the livers of rats fed on protein deficient diets It is definitely not choline nor is it the triglyceride content of the liver, which may vary considerably without changing the turnover of phospholipins The second question arises out of the fact that in protein deficient animals the losses of protein, phospholipin and ribonucleic acid from the liver are accompanied by losses of intracellular enzymes Thus the possibility has to be taken into consideration that the increased turnover of phospholipins and probably other cell constituents might eventually lead to an exhaustion of the liver cell

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DISCUSSION EFFECT OF LOW PROTEIN DIE[T] IN CIRRHOSIS

T C CHALMERS

With regard to the changes in the liver on low protein diet I want to talk about some work done by Drs Eckhart an[d] Davidson (1950) in Boston They took patients with fatt[y] cirrhosis acute fatty livers and put them on a diet devoid o[f] protein for as long as the patients were willing to take it usually about ten to twelve days It was with some trepida[-] tion that this was done but the patients seemed to withstand it well

Fig 1 shows a biopsy taken before the diet the second one No 8 is taken after ten days on a diet devoid of protein containing only about 1 gram of protein in a total of about 3 000 cals , and finally a repeat biopsy after the patient had been on a normal diet for about three weeks Histochemical studies were done It was found that the fat content did not change The protein content of the cell was determined grossly observing the blue staining ribonucleic acid granules in tissue fixed with Zenker s solution and stained with eosin methylene blue The granules decreased fairly markedly in the 10 days on a low protein diet suggesting that the protein content of the liver cells decreased and then went rapidly back towards normal when the patients were given protein The glycogen content increased when the patients were on a low protein diet

Fig 2 illustrates that the patients did very well on this diet devoid of protein They were in markedly negative nitrogen balance and their weights dropped to some extent The serum bilirubin came down from 9 to 2 mg per 100 cc the thymol turbidity changed only slightly the urine urobilinogen and

bile decreased quite markedly The patients became symptomatically much improved, their appetites returned, and they had to be put on a protein diet, not because they were sicker, but because they were craving better food

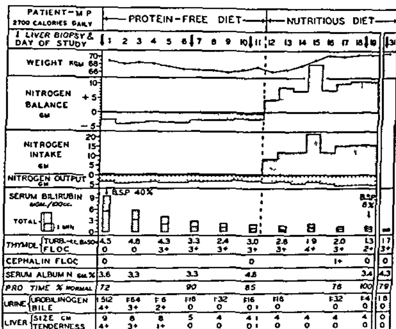


FIG 2 Effect of protein starvation and of a nutritious diet on nitrogen balance and liver function

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GENERAL DISCUSSION

S SHERLOCK Were these alcoholic patients ?

T C CHALMERS Yes

S SHERLOCK They had been taken off alcohol and put to bed ? *You had some controls ?*

T C CHALMERS Well, we had no controls who were continued on alcohol However patients on a good diet didn't get well any faster

effect," has been known experimentally for nearly twenty years, but [illegible]

point I made I never intended to deny that choline as such increased the turnover of phospholipins Our experiments were designed to test for a difference between diets with and without choline in the post absorptive state In one particular experiment, for instance we placed rats on an 8 per cent casein 40 per cent fat diet, with and without choline, for four weeks They were fed in the afternoon at 4 p m and any remaining food was removed every day at 9 a m After four weeks they were injected with ^{32}P at 9 a m and killed at 2 p m Under these [illegible]

in the post absorptive state there is no difference in the turnover of phospholipins due to the presence or absence of choline, of course, in no way contradicts the results of the numerous authors who found that injection of a single dose of choline increases the turnover of phospholipin for about ten hours

I would like to discuss another point, viz the effect of dietary protein on the fat content of the liver Some years ago we injected rats with carbon tetrachloride after they had been fed on different diets (Campbell and Kosterlitz, 1948) One group received a high protein diet, 70 per cent casein, for three days, the second an 18 per cent [illegible] Carbon

REFERENCE

Campbell, R M, and Kosterlitz, H W (1948) *Brit J exp Path*, 29, 149

DISCUSSION: GLUTATHIONE AND EXPERIMENTAL LIVER NECROSIS

O LINDAN and ELIZABETH WORK

Acute liver necrosis can be produced experimentally in rats by feeding diets deficient both in sulphur-containing amino-acids and tocopherol (see review by Himsworth, 1950) In our laboratory the condition can be produced in an average of 28 days (range 12–55 days) when weanling tocopherol-depleted rats are fed a "necrogenic yeast diet" (Lindan and Himsworth, 1951) This diet is deficient in tocopherol and contains only 7 per cent of protein, all of which is supplied by a baker's yeast deficient in cystine and methionine (Lindan and Work, 1951) Addition to the diet of cystine and methionine, or tocopherol will prevent the lesion

Since a deficiency of sulphur-containing amino-acids is one of the factors required for liver necrosis, one might expect to find a lowering of some sulphur fraction in necrotic livers Dent (1947) showed that there is no change in the cystine and methionine content of necrotic liver protein The only sulphur fraction of the liver known to vary with changes in dietary protein sulphur is the cystine containing tripeptide glutathione which was found by Leaf and Neuberger (1947) to be lowered during protein starvation and cystine deficiency We are therefore investigating the relation between necrosis and liver glutathione using the iodometric titration method (Leaf and Neuberger 1947) checked by paper chromatography

Normal rat livers contain about 230 mg per cent of glutathione, 92 per cent of which is in the reduced form When the rats are fed the necrogenic yeast diet, within nine days the glutathione level is found to have fallen to 84 mg per cent and now the reduced form represents only 70 per cent of the total (see Fig 1) Tocopherol supplements do not influence

this change in glutathione content produced by the yeast diet and the supplemented rats can live with this subnorma glutathione level in fairly good general condition In the

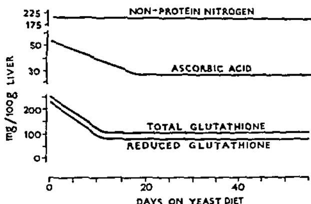


FIG 1 Effect of yeast diet on pre necrotic liver composition

bund rats show even lower glutathione levels (20 per cent normal) of which only half is in the reduced form (Fig 2)

This final fall of glutathione in the necrotic livers is accompanied by a sudden similar fall in non protein nitrogen, from about 200 mg per cent to about 100 mg per cent The fall in non protein nitrogen might be attributed to a dilution effect due to the acute swelling of the liver which accompanies necrosis, but the fall in reduced glutathione from the pre necrotic level is too great to be due solely to a dilution effect

Ascorbic acid levels in the livers were also estimated The livers of rats fed the necrogenic yeast diet showed a fall in liver ascorbic acid from 38 mg per cent to 26 mg per cent in twelve days and no further change was noted until necrosis occurred Necrotic livers contained practically no ascorbic acid

Supplementing the necrogenic yeast diet with cystine (0 6 per cent) prevented liver necrosis and kept the liver glutathione normal

At the time when liver necrosis develops and the glutathione and non protein levels are lowest, chromatograms of urine show the presence of a gross excess of amino-acids (including cystine) thus resembling those from cases of human

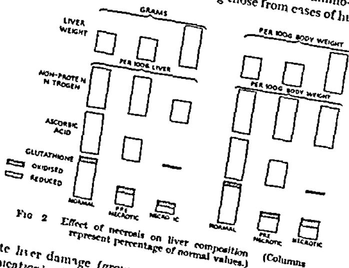


Fig 2 Effect of necrosis on liver composition (Columns represent percentage of normal values.)

acute liver damage (group A of Dr Dent s preceding communication) This sudden death of liver cells might result in release into the blood stream of glutathione and amino acids In the absence of the deaminating action of liver this might lead to a rise in plasma amino acids and consequent amino aciduria

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Lindan O and Himsworth H P (1950). *Brit J exp Path* 31 651
Lindan O and Work L (1951) *Biochem J* 48 337 and 344

this change in glutathione content produced by the yeast die and the supplemented rats can live with this subnorm glutathione level in fairly good general condition In th continued absence of tocopherol no further lowering of gluta thione is noted until the rat suddenly becomes ill and develop liver necrosis The necrotic livers removed from these mor

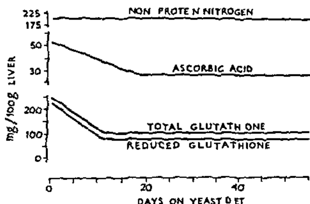


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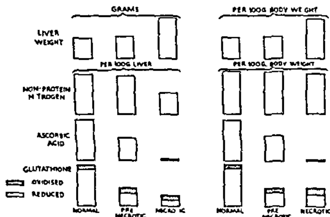


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DISCUSSION EFFECTS OF CYSTINE-DEFICIENT YEAST DIETS ON RAT LIVER

H FINK

In our laboratory we have been working for many years on the technical production of fodder or food, yeasts from materials such as wood sugar, sulphite pulp, and wort from the hydrolysis of straw Among other problems, we have been interested in trying to find out what is the value for animals and human beings of the protein produced by aerobic yeast culture *The results of this work, carried out in co operation* with Dr Hock and Prof Dr Dobberstein, have some interest in connection with liver injury

In 1943 we determined the biological value of the protein *of different yeasts by examining growing rats, using a method* similar to MacCollum's method The young animals which had a weight of about 50 g, all received a diet complete in regard to protein carbohydrate, fat, vitamins, minerals, etc, *only the kind and origin of the chief protein sources* varied The proportion of digestible protein to starch in cal ories was 1 to 10 We took milk protein (skim milk powder) as a standard, because it is known to be of full value In other cases we used protein of different yeasts, fungi, and other plants, in quantities equivalent to the nitrogen contents

As we had expected, the growth curves showed clearly that the protein of yeasts, fungi, and other plant material has a lower value The extent of this decrease was very different for different yeasts

Further, we were able to establish with a certain degree of assurance the reason for the lower value of the yeast protein We succeeded in showing that in the yeast protein, among the nine amino acids important for life, the sulphur containing ones are present in far too small a quantity With some

yeasts, beer yeast for instance, dietary supplements of 0 2 per cent cystine led to greatly improved growth curves so that these now equalled the growth curves for milk protein Recently, similar effects have been produced by supplements of methionine or keratin hydrolysate The yeast used for these experiments had been grown on sulphite pulp

Perhaps the observations which we were able to make on the yeast fed animals, which got no supplements of cystine, methionine or keratin hydrolysate, were of greater importance These animals fed almost exclusively with yeast protein or other proteins poor in cystine, not only remained behind in weight and growth but often died in greater numbers, so that at first we thought of intercurrent illnesses such as paratyphoid fever

Because there was little information to be had about pathological consequences of a diet deficient in cystine, we decided to study the chemical and histological changes in the animals, and for the purpose of studying the histological changes, Professor Dr Dobberstein came to our assistance

These histological experiments, which Dobberstein has published in detail in *Hoppe Seyler's Zeitschrift für physiologische Chemie*, revealed clear results It became obvious that, within the groups fed without cystine supplements up to 82 per cent of the animals which died during the experiment showed very characteristic changes in their organs Almost never did we observe these in animals fed on a diet which contained supplements of cystine

The most obvious changes took place within the liver, which appeared spotted and much, or little enlarged, besides dark red discoloured parts there were also found strikingly pale grey yellow sectors of varying size The consistence of the organ was friable Also the kidneys nearly always showed a pallor which was striking In some cases the spleen showed a strongly marked hæmorrhagic tumour No differences could be found in the other organs From the histological point of view, the liver changes were very characteristic A marked fatty degeneration of the capillary endothelium

takes place This is also found to some extent within the liver cells In the liver there was often necrosis without preceding fatty degeneration Simultaneously expanded hæmorrhages are to be seen in several lobes In some cases in which the liver degeneration apparently had taken place more slowly, an early cirrhosis was seen, as well as regenerative phenomena in the still surviving liver cells In the kidneys, degenerative changes in the endothelium were observed, as well as symptoms of glomerulitis, the changes are in the same direction as other authors have described in animals which were given excessive quantities of cystine

The resemblance of the findings in the sections both of liver and kidneys, to those of human eclampsia is striking It cannot yet be decided whether human eclampsia is an illness which comes from deficiency of cystine, because we are not quite certain about the nature of the accepted toxins in this condition At any rate it is striking that also pregnant rats cannot stand a diet deficient in cystine

In addition to this report, I want to add that our results are confirmed in essential points by K Ruppert, who has described his experiments in the Deutsche Med Wochenschrift 73, H 7/8, p 96 Further, P Gyorgy (6th Conf on Liver Injury, Josiah Macy, Jr, Foundation Conference May 1947, p 67) and K Schwartz (*Z physiol Chem*, **281**, 101, 109 (1944) have shown that our eclampsia of rats may be prevented by addition of small quantities of tocopherol to the diet

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DISCUSSION ACTION OF METHIONINE AND CHOLINE ON THE CHOLESTEROL CONTENT OF EXPERIMENTAL FATTY LIVER OF THE RAT

A GAJDOS

Best *et al* (1934) noted the increases in the levels of total and esterified cholesterol in dietary fatty liver Relatively little attention has been paid to these observations We have reinvestigated this problem and studied at the same time the action of methionine or choline on the cholesterol content of fatty liver (Benard *et al*, 1950)

A group of adult male rats has been fed on the following high fat, low protein diet casein, 4 to 6 per cent, sugar, 40 per cent, lard, 49 to 51 per cent, salt mixture, 5 per cent, and known vitamins in optimal doses Another group of rats, fed on wheat corn received every two days a subcutaneous injection of 0 6 ml carbon tetrachloride for ten days

Each group has been divided into three lots The animals of the first and second lots received respectively, during the whole experiment, a daily subcutaneous injection of 40 mg methionine or choline The rats of the third lot served as controls

Total cholesterol, free and esterified fractions were evaluated by the method of Delsal (1946)

As is shown in the table, total and esterified cholesterol were found to be increased in both dietary and toxic fatty livers Methionine or choline prevented these changes

The increase in esterified cholesterol in experimental fatty liver can be contrasted with the decrease in esterified cholesterol in the blood serum of patients with severe hepatic diseases The probable explanation is the different behaviour of *liver* cholesterolase and *serum* cholesterolase The first catalyses the hydrolysis of cholesterol esters, the second

Table I

	Rats treated with 40 mg. daily methionine					Rats treated with choline 40 mg. daily					Rats not treated with choline or methionine				
	Total liver lipid g. per 100g. wet tissue	Total cholesterol	Esterified cholesterol	Free cholesterol	Ratio: Esterified cholest. / Total cholest.	Total liver lipid g. per 100 g. wet tissue	Total cholesterol	Esterified cholesterol	Free cholesterol	Ratio: Esterified cholest. / Total cholest.	Total liver lipid g. per 100 g. wet tissue	Total cholesterol	Esterified cholesterol	Free cholesterol	Ratio: Esterified cholest. / Total cholest.
Carbon tetrachloride	8.2	0.154	0.038	0.116	0.25	6.7	0.218	0.034	0.184	0.23	11.8	0.177	0.06[illegible]	0.11[illegible]	0.37
	8.0	0.165	0.040	0.116	0.20	7.0	0.100	0.047	0.152	0.28	8.3	0.258	0.093	0.163	0.37
	6.5	0.130	0.020	0.110	0.15	6.9	0.17[illegible]	0.072	0.145	0.18	8.6	0.250	0.096	0.154	0.38
Average	7.6	0.150	0.036	0.114	0.23	6.8	0.200	0.044	0.153	0.20	9.8	0.228	0.095	0.143	0.37
Low protein high fat diet (3 weeks)	7.8	0.148	0.026	0.122	0.175	6.4	0.154	0.017	0.13[illegible]	0.11	12.7	0.227	0.107	0.120	0.47
	8.5	0.140	0.031	0.118	0.21	5.6	0.187	0.025	0.112	0.15	11.1	0.200	0.075	0.125	0.37
	6.9	0.170	0.010	0.160	0.06	7.6	0.183	0.045	0.138	0.24	14.8	0.259	0.120	0.138	0.46
						5.9	0.155	0.032	0.123	0.20	15.5	0.280	0.165	0.124	0.57
Average	7.7	0.136	0.022	0.133	0.160	6.4	0.157	0.047	0.127	0.185	13.8	0.243	0.116	0.127	0.46

catalyses the *esterification* of cholesterol The diminution of esterified cholesterol in fatty liver can be tentatively attributed to the decrease of the activity of liver cholesterolase Recently several workers have observed a similar decrease in the activity of different liver enzymes in rats fed on a low protein diet or treated with toxic agents (Yobling *et al*, 1915, Seifter *et al*, 1948, Westerfeld and Richert, 1949, Williams *et al*, 1949)

However it may be, we think that the modification of the cholesterol content in fatty liver may play a role in the mechanism of fatty infiltration of this organ The importance of cholesterol in the transport of fatty acids is indeed well established.

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DISCUSSION FLOCCULATION TESTS IN JAUNDICE

H DUCCI

The principal value of the flocculation reactions, empirically demonstrated derives from their contribution to the differential diagnosis of jaundice Their results are generally negative in post hepatic jaundice, and positive in the great majority of cases of hepato-cellular icterus

According to present knowledge regarding the mechanism of the flocculation tests (Kabat *et al*, 1943, Wunderly and Wuhrmann, 1947, Maclagan and Bunn, 1947) the serum is altered in hepato cellular jaundice in such a way as to give positive results The sera of normal individuals and of those with post hepatic jaundice, according to the classical view, [illegible]

high percentage of negative flocculation tests in post hepatic jaundice can be explained entirely on the basis of indemnity of the hepatic parenchyma We have repeatedly observed, in patients with biliary obstruction and negative flocculation reactions, the development of positive tests after spontaneous or surgical re-establishment of biliary flow to the intestines Moreover, in certain cases of prolonged post hepatic jaundice with negative flocculation tests hepatic biopsy has revealed clear cut damage to the liver (Ducci, 1948)

We have also observed frequently that the negative flocculations of sera from cases of post hepatic jaundice, left undisturbed in the laboratory, take longer to become positive than those of normal sera Moreover, the negative serum from cases of biliary obstruction may be stored for long periods of time in the refrigerator without developing the capacity to give falsely positive results, normal sera, on the other hand, acquire it in a short time

globulin, the thymol flocculation of the normal serum becomes positive, while that of the obstructive jaundice serum remains negative These results do not support Maclagan's belief that the flocculating capacity against the thymol reagent is exclusively related to hepatitis gamma globulin (Maclagan and Bunn, 1947)

Table I

Addition of Normal Gamma Globulin in Increasing Amounts to Normal and Post hepatic Jaundice Sera

Serum (ml)	Globulin* (ml)	Cephalin		Gold	Red	Thymol Turb	Thymol Flocc
			NORMAL				
0 5	0 05	(−)	+	3	1	6 0	(−)
0 5	0 10	(−)	+	4	3	7 3	(−)
0 5	0 15	(−)	++	5	4	8 7	(−)
0 5	0 20	(−)	+++	5	4	11 2	+
0 5	0 25	(−)	+++	5	4	13 3	++
0 5	0 30	(−)	++++	5	5	14 6	++
0 5	—	(−)	(−)	0	0	3 9	(−)
			POST HEPATIC JAUNDICE				
0 5	0 05	(−)	(−)	1	0	4 6	(−)
0 5	0 10	(−)	(−)	3	1	5 5	(−)
0 5	0 15	(−)	(−)	4	2	7 9	(−)
0 5	0 20	(−)	(−)	4	3	8 5	(−)
0 5	0 25	(−)	(−)	5	4	9 9	(−)
0 5	0 30	(−)	+	5	5	10 8	(−)
0 5	—	(−)	(−)	0	0	3 9	(−)

* 3 per cent solution

The inhibitory effect of alb...
hepat...

...demonstrated that in certain proportions... In these experiments gamma globulin produces a ...

Table II

VARIATIONS IN THE AMOUNT OF ALBUMIN ADDED TO CONSTANT MIXTURES OF NORMAL SERUM AND GAMMA GLOBULIN, AND OF POST HEPATIC JAUNDICE SERUM AND GAMMA GLOBULIN

Serum (ml)	Globulin* (ml)	Albumin† (ml)	Cephalin		Gold	Red	Thymol Turb.	Thymol Flocc
			NORMAL					
0 5	0 3	0 10	+++	++++	5	5	5 5	+
0 5	0 3	0 15	+++	+++	5	4	5 1	(−)
0 5	0 3	0 20	++	+++	5	4	3 9	(−)
0 5	0 3	0 25	++	++	3	3	3 1	(−)
0 5	0 3	0 30	+	++	2	1	2 7	(−)
0 5	0 3	0 35	(−)	+	2	1	2 6	(−)
0 5	0 3	—	+++	++++	5	5	7 4	+++
			POST HEPATIC JAUNDICE					
0 5	0 3	0 10	+	++	3	2	6 8	(−)
0 5	0 3	0 15	+	+	3	1	4 1	(−)
0 5	0 3	0 20	(−)	+	2	1	3 7	(−)
0 5	0 3	0 25	(−)	(−)	1	0	2 1	(−)
0 5	0 3	0 30	(−)	(−)	0	0	1 9	(−)
0 5	0 3	0 35	(−)	(−)	0	0	1 7	(−)
0 5	0 3	—	++	+++	5	5	10 8	(−)

* 3 per cent solution † 25 per cent solution

From the above observations it can be concluded that the serum of at least some cases of post hepatic jaundice with negative flocculation reactions, appears more resistant than normal serum to the precipitating effect of gamma globulin. In the case of normal serum, the negative flocculation tests are indeed related to the absence of qualitative or quantitative changes in its protein components. However, this explanation does not always hold for post hepatic jaundice sera. In certain cases of recent biliary obstruction, the negative flocculation reactions may reflect a normal liver parenchyma. But in some cases of post hepatic jaundice, with clear cut damage demonstrated by the pathological results of the true liver function tests, the serum possesses a special property

globulin, the thymol flocculation of the normal serum becomes positive, while that of the obstructive jaundice serum remains negative These results do not support Maclagan's belief that the flocculating capacity against the thymol reagent is exclusively related to hepatitis gamma globulin (Maclagan and Bunn, 1947)

Table I

Addition of Normal Gamma Globulin in Increasing Amounts to Normal and Post-hepatic Jaundice Sera

Serum (ml)	Globulin* (ml)	Cephalin		Gold	Red	Thymol Turb	Thymol Flocc
			NORMAL				
0 5	0 05	(−)	+	3	1	6 0	(−)
0 5	0 10	(−)	+	4	3	7 3	(−)
0 5	0 15	(−)	++	5	4	8 7	(−)
0 5	0 20	(−)	+++	5	4	11 2	+
0 5	0 25	(−)	+++	5	4	13 3	++
0 5	0 30	(−)	++++	5	5	14 6	++
0 5	—	(−)	(−)	0	0	3 9	(−)
			POST HEPATIC JAUNDICE				
0 5	0 05	(−)	(−)	1	0	4 6	(−)
0 5	0 10	(−)	(−)	3	1	5 5	(−)
0 5	0 15	(−)	(−)	4	2	7 9	(−)
0 5	0 20	(−)	(−)	4	3	8 5	(−)
0 5	0 25	(−)	(−)	5	4	9 9	(−)
0 5	0 30	(−)	+	5	5	10 8	(−)
0 5	—	(−)	(−)	0	0	3 9	(−)

* 3 per cent solution

The inhibitory effect of albumin is also greater with post-hepatic jaundice serum than with normal serum, as shown in the illustrative example in Table II In these experiments it is again demonstrated that in certain proportions normal gamma globulin produces a positive thymol flocculation, nevertheless, this effect is not seen with post hepatic jaundice serum, although thymol turbidity is increased

Table II

VARIATIONS IN THE AMOUNT OF ALBUMIN ADDED TO CONSTANT MIXTURES OF NORMAL SERUM AND GAMMA GLOBULIN, AND OF POST HEPATIC JAUNDICE SERUM AND GAMMA GLOBULIN

Serum (ml)	Globulin* (ml)	Albumin† (ml)	Cephalin		Gold	Red	Thymol Turb	Thymol Flocc
			NORMAL					
0 5	0 3	0 10	+++	++++	5	5	5 5	+
0 5	0 3	0 15	+++	+++	5	4	5 1	(−)
0 5	0 3	0 20	++	+++	5	4	3 9	(−)
0 5	0 3	0 25	++	++	3	3	3 1	(−)
0 5	0 3	0 30	+	++	2	1	2 7	(−)
0 5	0 3	0 35	(−)	+	2	1	2 6	(−)
0 5	0 3	—	+++	++++	5	5	7 4	+++
			POST HEPATIC JAUNDICE					
0 5	0 3	0 10	+	++	3	2	6 8	(−)
0 5	0 3	0 15	+	+	3	1	4 1	(−)
0 5	0 3	0 20	(−)	+	2	1	3 7	(−)
0 5	0 3	0 25	(−)	(−)	1	0	2 1	(−)
0 5	0 3	0 30	(−)	(−)	0	0	1 9	(−)
0 5	0 3	0 35	(−)	(−)	0	0	1 7	(−)
0 5	0 3	—	++	+++	5	5	10 8	(−)

* 8 per cent solution † 25 per cent solution

From the above observations it can be concluded that the serum of at least some cases of post hepatic jaundice with negative flocculation reactions, appears more resistant than normal serum to the precipitating effect of gamma globulin In the case of normal serum, the negative flocculation tests are indeed related to the absence of qualitative or quantitative changes in its protein components However, this explanation does not always hold for post hepatic jaundice sera In certain cases of recent biliary obstruction, the negative flocculation reactions may reflect a normal liver parenchyma But in some cases of post-hepatic jaundice, with clear-cut damage demonstrated by the pathological results of the true liver function tests, the serum possesses a special property

DISCUSSION: PROTEIN METABOLISM IN LIVER DISEASE

M. BJØRNEBOE

I would like to show some figures demonstrating the effect of ACTH on serum proteins in a case of cirrhosis of the liver. The patient was a 45-year-old man with a history compatible

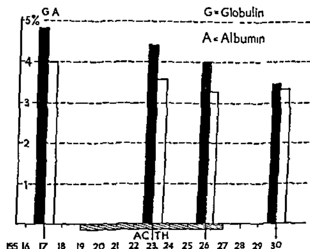


FIG. 1 Serum globulin and serum albumin in a patient with chronic hepatitis before, during and after treatment with ACTH 100 mg a day

with cirrhosis of the liver of almost two years' duration. Repeated serum analyses during this time showed a globulin concentration constantly about 5 per cent. The diagnosis of chronic hepatitis was confirmed by biopsy. The graph (Fig. 1) shows his serum proteins during and after treatment with ACTH 100 mg. a day for eight days. It is evident that the globulin concentration and the relation of globulin to

total protein decreases Protein fractionations were done with

This has been shown before Dr F Hanger in New York has been kind enough to demonstrate the same effect to me on a patient treated with cortisone

Now I would like to suggest an explanation of this effect Together with E Fischel and H Stoerck we have been able

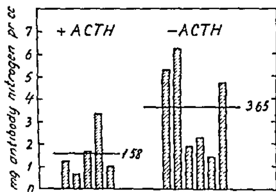


FIG 2 Antibody protein nitrogen (mg per ml of serum) in individual rabbits after 28 days of immunization with and without treatment with ACTH

(From M Bjorneboe E Fischel and H Stoerck The effect of cortisone and adrenocorticotrophic hormone on the concentration of circulating antibody *J exp Med* in press)

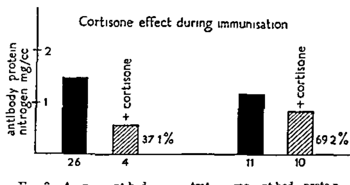
to prove that in rabbits the formation of antibody globulin is inhibited by ACTH and cortisone Figs 2–4 will give you an impression of this effect In Fig 2 you will see antibody levels in rabbits treated with ACTH during immunization as compared with controls Then (Fig 3) you see the

4) you see

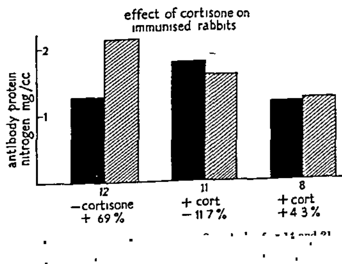
In these

unization

does *not* increase as compared with controls



(M Bjørneboe, E Fischel and H Stoerck *loc cit*)



(M Bjørneboe, E. Fischel and H Stoerck *loc cit*)

These experiments may have some interest in connection with the experiences mentioned before with chronic hepatitis [illegible]

have shown that in chronic hepatitis there is an increase of plasma cells in the bone marrow We have in a recent paper suggested that this may be interpreted as a sign of intensive *antibody formation*, as we and several others have shown that there is a positive correlation between antibody formation and occurrence of plasma cells It is possible that the antibody in question is an antibody to hepatitis virus or a ' liver damage antibody" as Eaton has suggested, an antibody arising from damaged liver acquiring antigenic properties in the organism to which it belongs

Conclusion The effect of the suprarenal cortical hormones on the serum proteins in chronic hepatitis may be explained as an inhibiting effect on the formation of antibody globulin

DISCUSSION ELECTROPHORETIC TITRATION OF SERUM ALBUMIN IN INFECTIOUS HEPATITIS

N H MARTIN

Fig 1 shows an electrophoretic titration of the serum albumin and beta + gamma globulin in a case of infectious hepatitis I want to stress that this serum was taken,

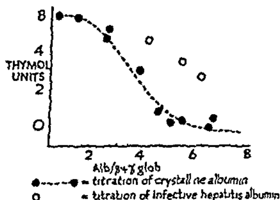


Fig. 1 Effect of albumin in inhibiting positive thymol effect of $\beta+\gamma$-globulin in infective hepatitis with (a) normal crystalline albumin and (b) albumin from infective hepatitis.

stored at 0°C, dialysed immediately against phosphate buffer, reduced to the necessary concentration for isolation, and then removed by electrophoresis We have been rather anxious *about intimate changes in proteins when other* techniques are used This is an old illustration, but the newer data fit these curves reasonably well There is a qualitative difference in the two albumins, if you use thymol units titrated against albumin, balanced against the beta- and gamma globulin which were also isolated electrophoretically It seems to us that there is a real qualitative

difference in the albumin in infectious hepatitis in what I want to call the "specific" turbidity reactions, especially the thymol reaction We believe that these curves are strong evidence of it because we have been particularly careful not to damage the albumin

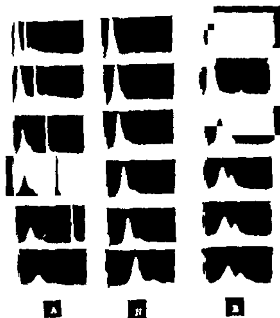
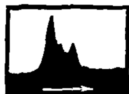


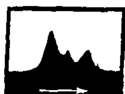
Fig 2 Ultracentrifugal diagrams showing fractions with high sedimentation constants in serum from a case of reticulosis (A) and advanced liver disease (B) Normal serum (N) is shown for comparison

Now the non specific reactions Fig 2 is an ultra centrifugal study Here "N" is the normal ultra centrifugal diagram showing the usual balance of components "B" is an ultracentrifugal run from cirrhosis, showing an increase of the normal heavier components That little hummock is a heavy

component which runs ahead You do see traces of heavy component occasionally in normal sera, but it seems to occur in the normal sera which give slightly high flocculation tests "A" is one with a great deal of heavy component of two types It, of course, gave strong positive reactions with all the flocculation tests

It is perfectly obvious that if you get a component like that circulating in the serum, it's going to flocculate if you


(1)


(2)


(3)


(4)

FIG 3 Electrophoretic Analysis Arrows indicate the direction of migration.
(1) Descending Limb 1 hour
(2) Descending Limb 2 hours
(3) Descending Limb 3 hours
(4) Ascending Limb 3 hours

give it half a chance We've stripped it off and examined it I have no really accurate diffusion constants on it, but it's of the order of a million, and is not rich in lipoid

Fig 3 gives an example from a chronic cirrhotic I rather like this because it's such a pretty control, both patterns are running at the same time, and you've got your ascending limb as control for your descending limb There is spontaneous precipitation of the gamma globulin "in the descending limb

three hours." This again I think is a non-specific reaction. I've seen it in cases of nephritis, and other cases in whic there are unusual flocculation tests.

GENERAL DISCUSSION

[illegible]

albumin ?

[illegible]

[illegible] a quart[illegible] of a century ago Dr Mann and I undertook so physiological studies of the liver from which we hoped to develop clinical test of liver function The only one which seemed to offer a possibility was the changes in purine metabolism, particularly u acid which take place on removal of the liver [illegible]

could take care of all the action of the liver that *could be tested by any* methods which we had at that time And indeed I do not know of any since

The one test which we have come to rely upon as a strictly *functional* test of the liver is the bromsulphalein test Being a laboratory man, I am convinced that one of the great difficulties with the reporting of all of these tests is the inaccuracy of the method employed Laboratory

unless you have those technicians under strict and absolute control and unless the tests are done exactly In our bromsulphalein work we inject 5 mg per kg of body weight because 2 mg does not cause sufficient stress on the liver to show clinical results One must wait a certain period of time, and that period of time must be exact We wait one hour, and any retention of over 6 per cent is considered to be abnormal There are many reasons for this selection I would like to

T B MAGATH As far as I know, the bromsulphalein test has practically no value in the presence of jaundice The jaundice patient will

years duration

liver function tests and it is a matter of convenience that t[illegible] come to be so regarded Positive results can be caused by a number of non hepatic conditions and I think we are under *no delusions on* that particular point

F WUHRMANN We believe that all the so called 'liver function tests involving the blood protein alterations are *non specific*, but their clinical value is nevertheless well established in regard to the whole clinical picture In plasma protein metabolism of the blood serum

under pathological conditions there is always an inverse unilateral regulation mechanism that is in all diseases with no exception there is always a decrease of albumin and a simultaneous increase of the globulins and never the contrary We think that this fundamental biological law is one expression of the 'adaptation syndrome' of Selye

Besides qualitative alterations in the albumins and gamma globulins in cases of acute infective hepatitis, I think there are also alterations in the beta$_1$ globulin fraction In such cases we found that the only proteins which showed an abnormal level in our cadmium reactions were the beta$_1$ globulins i e increased beta$_1$ globulin in an altered serum prohibits the turbidity in the cadmium reaction (above all in cases of beta$_1$ globulin myelomas)

PART II

THE ÆTIOLOGY OF HEPATIC CIRRHOSIS

Chairman : G. R. Cameron

EXPERIMENTAL HEPATIC CIRRHOSIS

L. E. GLYNN

I feel somewhat at a disadvantage compared with most of the other participants in these discussions because for the past three years I have ceased to take an active part in research on liver disease.

The subject we are discussing this afternoon is the ætiology [illegible] name cirrhosis

The first of these is repeated exposure of the liver to toxic agents of the type of carbon tetrachloride, single injections of which produce zonal necrosis of the liver If you repeat these injections at sufficiently short intervals of time, the liver eventually becomes extensively scarred and granular and resembles very closely the typical appearance of Laennec's cirrhosis.

The second type of procedure which gives rise, experimentally at least, to diffuse scarring of the liver is to produce severe fatty infiltration of the liver by dietary deficiency of lipotropic agents If this fatty infiltration is maintained for a sufficient length of time, fibrosis gradually develops, and ultimately reaches such a degree that again the picture closely resembles that of Laennec's cirrhosis (Fig. 1).



FIG 1 Three livers showing the evolution of diffuse hepatic fibrosis Rats maintained on a diet deficient in lipotropic factors The earliest stage shows accentuation of the lobular pattern due to the deposition of fat and the slight granularity of commencing fibrosis The intermediate stage shows increasing granularity and several more conspicuous regeneration nodules In the advanced stage considerable deformity and nodularity are apparent Two thirds natural size

The third type of procedure is to produce massive necrosis of the liver by a diet deficient in thio amino acids and tocopherol If the animals do not die of the acute phase of the disease they recover and show extensive coarse scarring in place of the areas of necrosis with nodular proliferation of the surviving liver cells a condition which is termed post necrotic scarring and nodular hyperplasia one form of cirrhosis of the liver

The fourth procedure which is associated with hepatic cirrhosis is biliary obstruction with or without infection of the biliary tract

I propose to take the first three of these procedures and to discuss what in my opinion are some of the problems which are still unsolved with respect to each of them

Repeated exposure of the liver to toxic agents such as carbon tetrachloride is the simplest method of producing cirrhosis of the liver It is doubtful whether this particular procedure plays any great part in the genesis of cirrhosis of the liver in human beings although with the further industrialization of most of the world and with the further introduction of toxic solvents into industry it is probable that we shall see more examples of this type of hepatic pathology Of the unsolved problems of this type of cirrhosis the first is that we know almost nothing of *how* these particular toxic agents produce the necrosis of the liver which ultimately leads to the fibrosis and particularly we are ignorant of why certain zones of the liver are selected for their apparent toxic activity The majority of these toxic agents produce their necrosis at least primarily in the centrilobular area of the liver that is in the areas around the central veins It isn t at all clear why they do this

It is possible that anoxia plays some part in affecting the susceptibility of the liver cells to toxic agents From what we know of the circulation through the liver it is reasonable to suppose that the cells most remote from the portal tract would be those exposed to the lowest oxygen tension and it may well be that there may be certain critical levels of oxygen

tension above which liver cells are resistant to such poisons as carbon tetrachloride

The other possibility is that these toxic agents themselves seriously interfere with the circulation through the liver, for example by producing gross swelling of the liver cells. Professor Himsworth and I (Glynn and Himsworth, 1948) thought we could demonstrate this by injections of Indian ink into the spleen, and thus show the compression of the liver sinusoids by the swollen liver cells but that was not completely satisfactory since the injection technique is itself not free from criticisms, to which it has already been subjected by Professor Cameron Even if it be true we are still ignorant of the actual mechanism by which these poisons cause the swelling of the liver cells sufficient to impede the circulation through the sinusoids

The third problem still unsolved in the genesis of this type of cirrhosis is the actual site of the subsequent scarring which occurs It was for many years thought that the scarring in carbon tetrachloride cirrhosis was primarily periportal whereas it would appear from more recent work (Ashburn *et al* 1946) that it is at least primarily, mainly around the central veins and that the portal tracts become involved much later

The fourth problem which is still unsolved is the question of reversibility of this type of cirrhosis Many years ago Professor Cameron and Professor Karunaratne (1936) studying hepatic cirrhosis produced by repeated injections of carbon tetrachloride made the very interesting observation that even when an apparently advanced degree of cirrhosis was reached, if the injections of carbon tetrachloride ceased and the liver in the animal allowed to continue undisturbed then the liver eventually returned completely to normal, and all traces of previous cirrhosis disappeared, but if the injections were continued beyond a certain critical time, then subsequent cessation of the treatment did not allow the liver to return to normal So there was a certain stage in the evolution of this type of hepatic cirrhosis at which

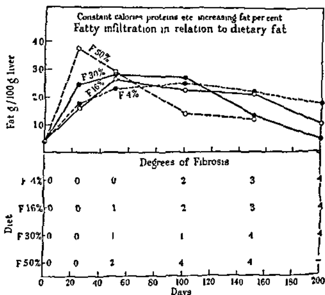
the condition became irreversible It is not known what determines this particular critical stage, but it is at least possible and suggestive that it depends upon the degree of alteration of the hepatic circulation There is, as you know, a considerable reduction of circulation through the liver as cirrhosis progresses, and if, as seems highly probable, reversibility depends upon an adequate blood supply to the liver, then a stage will eventually be reached when the degree of fibrosis in the liver will so reduce the circulation through it that we might reasonably expect the condition to become irreversible

Those then are the problems as I see them, which still remain unsolved with respect to that particular type of cirrhosis

The second type of cirrhosis is that which follows prolonged fatty infiltration in the liver This is a ready and reliable method of producing diffuse fibrosis in experimental animals, and has been used successfully in rats, mice, guinea pigs, dogs and rabbits, and is almost certainly a sequence which does occur in human beings Whether it is the invariable sequence in the evolution of diffuse fibrosis, I am not prepared to say but it is quite beyond doubt that at least in other parts of the world where nutritional levels are extremely low that fatty infiltration is definitely a stage in the evolution of diffuse hepatic fibrosis

Here again the mechanism by which the fatty infiltration leads to cirrhosis is still problematical and, to some extent, there is considerable difference of opinion Professor Himsworth and I (Glynn, Himsworth and Lindan, 1948) are inclined to the view that the evolution of this type of cirrhosis depends on impairment of the circulation through the sinusoids of the liver which are in this condition, compressed by the distended liver cells In accordance with this view is the observation that there seems to be a critical level of fat below which fibrosis will not occur (Figs 2 and 3) Moreover, not only an excess of neutral fat in the liver, but of many other substances, may lead to progressive cirrhosis, e g cholesterol, glycogen and methyl cellulose

The chief objection to the view that fatty infiltration acts in a mechanical sort of way, compressing the sinusoids, is perhaps Handler's observation (Handler and Dubin, 1946)



cirrhosis
(*Brit J. exp Path*, 1948, 29, 1)

cannot go into, but one which I think Handler himself has suggested

thyroid,

less likely

the metabolic demand of the liver cells is reduced under the influence of deficiency of thyroid.

I will just briefly refer to the third type of experimental cirrhosis, that which follows massive necrosis of the liver. It requires a joint deficiency of thio amino acids and tocopherol. The problems which remain to be elucidated in this particular type of necrosis and cirrhosis of the liver are: firstly, is the necrosis due to the production of some toxic material within

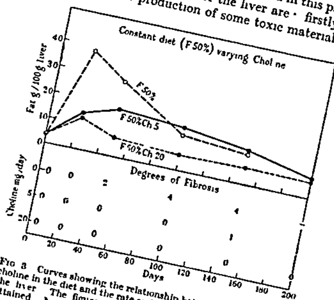


FIG 8 Curves showing the relationship between the amount of choline in the diet and the rate and degree of fatty infiltration of the liver. The figures below indicate the degree of fibrosis attained. Note the critical level of fatty infiltration of about 15 per cent below which fibrosis is not induced.
(*Brit J exp Path*, 1948, 29, 1)

the body, or to some toxic material introduced into the diet? The observations seem to refute both those possibilities, in that there is no evidence whatever that the diet contains a positively toxic substance, and our observations on the distribution of the necroses in the liver, that they are maximal where the liver is exposed to pressure from neighbouring organs would suggest that we are dealing with a deficiency

rather than the positive presence in the blood stream of some toxic agent

The second problem is how do Vitamin E and sulphur containing amino acids protect the liver ? What do they have in common, in that either of them is adequate to protect the liver in the presence of a deficiency of the other ?

Finally, an observation which is extremely difficult to explain, why is it that if you allow the protein intake in the diet to fall below a critical level (in our experiments it was 200 mg of casein per rat per day), you cease to get massive necrosis of the liver ? We found (Himsworth and Glynn 1944) that with levels of between 500 and 200 mg you get massive necrosis, but not if the intake falls below 200 mg That leads to extremely interesting theoretical speculations which I hope we have time to consider further in the discussion

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EFFECTS OF ANTIBIOTICS AND VITAMIN B_{12} IN CIRRHOSIS AND NECROSIS OF THE LIVER

P GYÖRGY

I WISH to reverse the usual procedure and start with the summary

Fig 1 is a summary of the effect of dietary factors in cirrhosis and in necrosis Today I wish to refer to only two the inclusion of antibiotics which of course are not really dietary factors, and Vitamin B_{12}

DIETARY FACTORS IN LIVER INJURY

	CIRRHOSIS	NECROSIS
PROTEIN	BENEFICIAL	BENEFICIAL
METHIONINE	BENEFICIAL	BENEFICIAL
CYSTINE	INJURIOUS	BENEFICIAL
CHOLINE	BENEFICIAL	NO EFFECT → INJURIOUS
VITAMIN E	NO EFFECT	BENEFICIAL
DIETARY FAT	INJURIOUS	NO EFFECT → INJURIOUS
VITAMIN B_{12}	BENEFICIAL	NO EFFECT
ANTIBIOTICS	?	BENEFICIAL

FIG 1

As you see antibiotics have been referred to here as beneficial factors in the prevention of massive dietary necrosis The reasoning which led us to antibiotics was as follows Glynn pointed out in his discussion that sulphur-containing amino acids and Vitamin E are beneficial factors They have nothing in common in a chemical sense sulphur-containing amino acids are water soluble, Vitamin E is fat soluble

However, they have a pharmacological similarity, they are both known to be detoxifying agents Therefore, we assumed that they may detoxify some toxic metabolite or *toxic product* in the body which is perhaps to some extent responsible for the *massive necrosis*

At that time Himsworth and Glynn claimed that the greater incidence of necrotic changes in the left lobe has some connection with the dual blood supply Today I notice Dr Glynn is preferring the pressure theory for the left lobe, but maybe I am mistaken I was very much impressed by this explanation of the dual blood supply, namely that the left lobe gets *the blood from the spleen, large intestine* and the stomach, and the right lobe from the small intestine and the ascending colon Very ingeniously Himsworth and Glynn suggested that the preference of the necrotic change for the left lobe is due to the fact that from the small intestine sulphur containing amino acids are absorbed and are protecting the right lobe I thought that perhaps the reverse is true, that *some toxic substance is reaching the left lobe These toxic* substances might come from the large intestine

At that time Stokes was studying the clinical aspects of hepatitis, and was using aureomycin With aureomycin in acute hepatitis no effect was noticed, but in chronic or sub acute hepatitis and in hepatic coma it proved to be beneficial He completely independently, felt that maybe the aureomycin affects the intestinal flora, preventing their metabolites from reaching the liver injured by the virus, and therefore aggravating *the condition We joined forces with him, and tested* this concept on experimental massive necrosis

We used the usual yeast diet of *Himsworth and Glynn*, which causes necrosis in practically all the animals We had 20 controls, and 22 animals on the same diet with the addition of aureomycin (50 mg daily mixed with the diet) After 160 and 170 days 95 per cent of the aureomycin treated rats *were still alive*, and only little more than 10 per cent of the control rats (Fig 2) Therefore the aureomycin had a definite *protective effect on the production of massive necrosis*

In a subsequent study, we tested several antibiotics on slightly younger rats In the first experiment the rats had an initial weight of 90–100 gm, whereas in this and the following set of experiments the weights were 45–55 gm All the controls died in less than 50 days, those with the aureomycin petered out slowly, and finally all died, but there was a statistically very significant delay A statistically

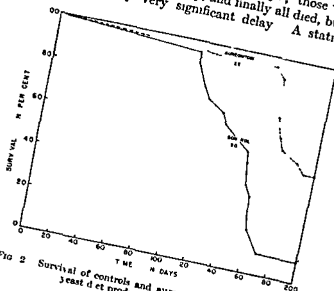


FIG 2 Survival of controls and aureomycin treated rats on yeast diet producing liver necrosis

significant delay was obtained with streptomycin but not with polymyxin (Fig 3)

Fig 4 shows the same type of experiment with vitamin B_{12} and aureomycin *Streptomyces* produces both aureomycin and B_{12} and since our yeast diet does not contain B_{12} we had to exclude the possibility that aureomycin acts, not as an antibiotic, but as an involuntary source of B_{12} At this time a paper appeared in the surgical literature showing that the anti bacterial effect of streptomycin on the intestinal

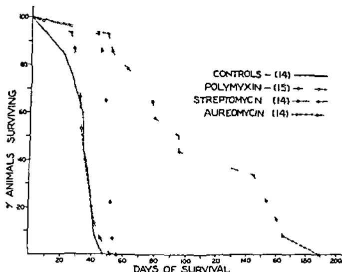


FIG 3 Comparison of aureomycin streptomycin and polymyxin in delaying death from liver necrosis in rats

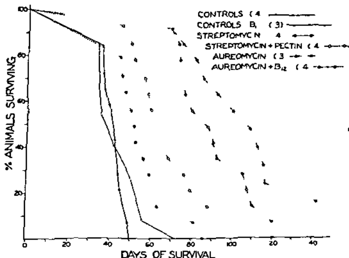


FIG 4 Comparison of aureomycin streptomycin and vitamin B_{12} in delaying production of liver necrosis in rats

flora was enhanced by the addition of pectin. Therefore we tested the effect of streptomycin plus pectin. As you see B_{12} had no effect on the production of massive necrosis and had no statistically significant effect on the aureomycin delaying effect. Streptomycin delayed necrosis and streptomycin plus pectin delayed it even more.

In the last experiment to be reported (Fig 5) some other antibiotics were tested, chloromycetin, sulphaguanidine,

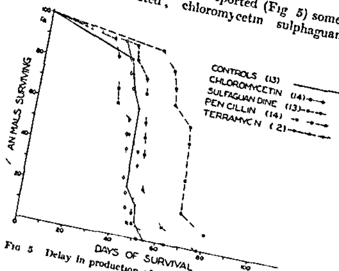


FIG 5 Delay in production of liver necrosis in rats by antibiotics

penicillin and terramycin. Statistically significant delays in the production of massive necrosis were produced by only sulphaguanidine and terramycin.

To summarize, the most effective antibiotic was aureomycin, second best terramycin, third streptomycin and then sulphaguanidine. Polymyxin, penicillin and chloromycetin were ineffective.

In addition to the protective or delaying effect on massive necrosis all the antibiotics which we used had an effect on

weight The animals r-- --
(Fig 6), although the di
ly not a normal one, bu
with the same caloric intake, the difference is statisticall
significant

The effect of other antibiotics, such as streptomycin polymyxin, chloromycetin, terramycin, penicillin as well a

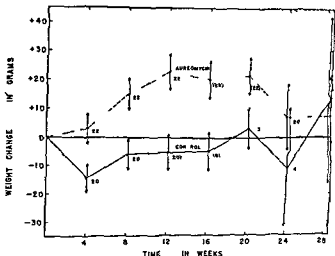


FIG 6 Effect of administration of aureomycin on body weight of rats on diet producing liver necrosis

of sulfaguanidine on weight gain is summarized in Tables I–III All these antimicrobial agents appeared to stimulate weight gain, which, although not very marked, was significantly superior to that observed in the untreated control groups Further, B_{12} had no, or only slight, potentiating effect on weight gain

Weight gain under the influence of aureomycin has been observed, not only on the necrogenic yeast diet, but also in animals receiving aureomycin supplements to the usual

Table I

Exp. 48		Average Weight Gain (g) 1–4 weeks
Control	(13)*	4.4 ± 1.3
Aureo	(14)	24.0 ± 1.05
Poly	(15)	12.0 ± 1.05
Strep	(14)	25.5 ± 0.9

*Number of animals

Table II

Exp. 50		Average Weight Gain 1–4 weeks
Control	(14)	7.0 ± 1.1
Control + B_{12}	(13)	10.5 ± 1.0
Aureo	(13)	27.0 ± 1.22
Aureo + B_{12}	(14)	23.6 ± 0.85
Strep	(13)	12.5 ± 0.8

Table III

Exp. 51		Average Weight Gain 1–4 weeks
Control	(13)	6.2 ± 2.0
Chloromycetin	(13)	16.0 ± 1.15
Sulfaguanidine	(13)	14.8 ± 1.5
Terramycin	(14)	18.7 ± 1.03
Penicillin	(14)	17.6 ± 1.15

low protein high fat diet which leads to cirrhosis. Ten males fed the basal experimental diet lost, during the experimental period (100 days), 41.2 g (S.E. ±11.0); ten males on the same diet *plus* aureomycin gained during the same time 16.3 g (±6.5). The corresponding figures for two groups

of female rats were $-34\ 8$ g ($\pm 6\ 4$) in the control group, and $+19\ 6$ ($\pm 3\ 6$) in the aureomycin group These differ nces between the weight figures in the control and aureo nycin groups are statistically highly significant

We may summarize by saying that some of the antibiotics have an effect on *prevention of massive necrosis, all have an* ffect, at least temporary, on weight gain Obviously we can only speculate about these effects Our present theory is hat the effect at least in part is through suppression of the ntestinal flora preventing toxic metabolites of the intestinal lora from reaching the left lobe of the liver, and therefore preventing necrosis The fact that it is a delaying effect fits n very well with our concept, because delay means that it is not a complete protection *like that of cystine or methionine* r vitamin E The antibiotics depress bacterial growth ut the bacteria finally become resistant, and the resistant acteria produce toxic substances and eventually cause ecrosis

Our theory is being put to the crucial test in the germ free aboratories of Notre Dame Professor Reyniers and his roup are feeding germ free animals with the necrogenic east diet *If our theory is right they will not come down with* assive necrosis

It is questionable that superiority of aureomycin over all her *antimicrobial agents in the delay of dietary massive* crosis may be explained only by its effect on the intestinal ora An additional systemic effect is a distinct probability

Finally a few words on the lipotropic effect of B_{12} In a cent paper (*Proc Soc exp Biol Med*, **73**, 372, 1950) we monstrated the lipotropic effect of vitamin B_{12} in rats fed low protein low fat diet The same supplement of crystalline tamin B_{12} ($0\ 5\gamma$) had no lipotropic effect when given to ts kept on a low protein high fat diet In an experiment st completed we found that the lipotropic effect of crystal ne B_{12} became evident even on a low protein high fat diet ovided *much higher doses of vitamin* B_{12} were used (20γ ily) The findings are summarized in Table IV

The lipotropic effect of vitamin B_{12} may attain important practical significance in the management and prevention of the so called "fatty liver disease," widespread over a large tropical belt of the world

Table IV
LIPOTROPIC EFFECT OF B_{12}
♀ Rats Av 154 g (142–175)
B_{12}–20γ, Meth–15 mg, daily

Group	Food g d	% Wt Chg	Wt	Liver % Fat
Cont.	8 4	−1 4	6 9	25 1±1 6
B_{12}	8 8	+3 8	5 8	10 0±1 0
Meth	8 6	+4 9	7 3	19 9±1 6
$M+B_{12}$	9 6	+6 2	6 2	7 3±0 4

GENERAL DISCUSSION

L. E GLYNN Dr Himsworth and I haven t altered our views on circulation My remarks about the effects of pressure are in addition to the effect of circulation

The very interesting experiments by Dr György on the effects of antibiotics do not necessarily imply that toxic agents come from the large bowel We don t know anything really about the metabolic activity of these powerful antibiotics. Some of the sulphonamides exhibit an anti thyroid effect and some of the antibiotics an anti oxidant effect so that it is conceivable that some of them have a somewhat similar action to the tocopherols in preventing massive necrosis.

P GYÖRGY It would be very unusual for these antibiotics systemically completely different to have the same metabolic effect for instance an antioxidant effect I have tested them and they do not have an anti oxidant action On the other hand I do not deny and am even inclined to believe that there may be an additional systemic site of action

HISTOLOGICAL STUDIES ON FATTY INFILTRATION OF THE LIVER IN CHOLINE-DEFICIENT RATS

W S HARTROFT

In the course of observations on Wistar rats fed low choline diets for periods up to one year or even longer, we have had the opportunity to study the morphological changes and the pathogenesis of lesions developing in the livers of these animals The experiments were conducted by Professor C H Best, Dr E A Sellers, Dr J H Ridout and the author at the Banting and Best Department of Medical Research of the University of Toronto We observed the development of the fibrous lesions and reported on those some time ago (Hartroft, 1950) In the course of this investigation it became apparent that the fat which accumulated in the liver and preceded the development of fibrous tissue may be located either *within* the cell, e g intracellularly, or later as the disease progresses, this intracellular fat may become extracellular This concept of the two types of fat distribution—intracellular versus extracellular—is, we think, of importance in considering the ætiological factors responsible for the deposition of fibrous tissue in the liver, and is of perhaps even greater importance in interpreting the effects of lipotropic factors restored to diets of choline-deficient animals or man

Fig 1 In the early stages of fatty change in the liver, small droplets of fat appear within the cytoplasm of each cell These droplets rapidly coalesce into single large vacuoles in the liver cells of rats fed a diet low in choline for two or three days

Fig 2 The cytoplasm of each cell is then filled with one or two large spherules of stainable fat

Fig 3 If the animals are maintained on a low choline diet for one or two months, many of the intracellular fat droplets are released from their parent cells by a process of rupture The fat, now extracellular, is contained within the lumen of a cyst The walls of the cyst are formed by the cells from which the fat had escaped

Fig 4A is a section from the liver of a normal rat, fed the basal diet supplemented with choline In Fig 4B from the liver of a rat fed the basal diet (without choline) for ten days, large intracellular droplets of fat each confined to a single cell are seen In Fig 4C from the liver of a rat fed the low choline diet for 83 days, these fatty spaces are relatively enormous Liver cells in the control animal (Fig 4A) measured 14 micra in diameter in a paraffin section, cells distended by single fatty spaces (Fig 4B) are 16–18 micra in diameter while the fatty spaces shown in Fig 4C are 30 to 35 micra in diameter and others are over 60 micra In the liver of a rat fed the diet for 65 days the diameter of such a space may reach 100 micra in the paraffin sections The cellular membranes separating the intracellular spherules of fat rupture and the spherules then fuse to form single large pools of fat which are surrounded by the cells from which the fat originally escaped This fat is now extracellular, although surrounded by its parent cells which surround it and wall it off in the form of a cyst

Fig 5 The distribution of the fatty cysts is shown in this illustration to be centrolobular Such a distribution is similar to that of the fibrous tissue which develops later The non portal locus of this type of fibrosis has been previously demonstrated by others (Lillie *et al*, 1942, Glynn *et al* 1948)

If a single cyst is examined at a higher magnification it is seen that the nuclei of the cells which form the wall of such a cyst are numerous As many as 60 to 80 cells have been found in the wall of a single fatty cyst which was subjected to serial sectioning As the animals are maintained for longer periods on a low choline diet these large cysts begin to atrophy

Fig 6 provides an example of this type of atrophy The shrunken cysts are surrounded and partly replaced by strands of blue staining (dark in photograph) fibrous tissue The fibrous tissue which replaces the cysts has the same distribution as did the fatty cysts This produces an annular fibrosis of a non portal nature which links together neighbouring centrolobular regions As shown in Glynn's illustrations and also with our animals, after fibrosis has developed the fatty content of the liver decreases even though the animals are still maintained on the basal diet This is associated with the atrophy of the fatty cysts, for the lipid they contained leaves the liver The pathways by which the fat escapes will be considered in the last section of this paper

First I would like to show with the aid of a series of slides what happens not only to the intracellular fat, but more particularly to the extracellular fat in the liver when choline is restored to the diet of an animal previously fed a food mixture low in the lipotropic factors

In Fig 7 the liver fat is all in the form of small intracellular spherules, none is extracellular for fatty cysts have not yet formed The illustration is from the liver of a rat fed a low choline diet for only seven days If choline is restored to the diet of such an animal, in three days the demonstrable fat in the liver is almost completely wiped out as shown in Fig 8

Fig 8 represents a section from the liver of an animal similar to that shown in the preceding illustration, sacrificed two days after restoring choline to the diet Only a small amount of stainable fat has persisted in those cells which surround the central vein

If such an animal is not sacrificed until three days after restoring choline to its diet only a few drops of stainable fat around central veins can be found, and these areas are found only with difficulty for most of the liver is completely free of stainable fat Dietary choline can mobilize with extreme rapidity stainable fat which has accumulated in the livers of rats which have previously been fed diets low in

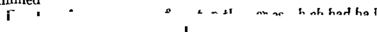
lipotropic factors for periods of 7 to 10 days This is only true however if the excess fat which has accumulated in the liver is *intracellular* in nature Under similar conditions, *extracellular* fat behaves somewhat differently under the influence of dietary choline The next series of figures will illustrate this

In Fig 9 is illustrated the appearance of the liver of a rat fed a low choline diet for approximately one year Many large fatty cysts may be seen surrounding a fibrous trabecula on the left Smaller droplets of intracellular lipid are present at the edges of the low power field, and between the fatty cysts in the high power field on the right

As shown in Fig 10 when a comparably choline deficient rat has choline restored to its diet and is killed three days later, all the *intracellular* fat has disappeared, just as in the case of a rat which had been choline-deficient for only seven days But the *extracellular* fat contained in cysts persists This is shown in the high power view on the right The surrounding cells which have lost their intracellular fat have an abnormal appearance in that their cytoplasm is palely staining and vacuolar

If a rat is fed the low-choline diet for one year, followed by a choline supplemented diet for a period of eight days, all intracellular stainable fat disappears, and in high power the cells which have been cleared of fat are seen to have lost their vacuolar appearance and appear normal The fatty cysts have persisted but are beginning to show changes which might be described as degenerative Fig 11 represents the appearance of a paraffin section from such a liver The persisting fatty cysts are shown and these resemble similar structures which have been illustrated in liver biopsies of alcoholics treated with lipotropic substances The fatty cysts in this choline-deficient rat treated with dietary choline for eight days have persisted As the life span of a human is 20 times that of a rat, one might on this basis expect fatty cysts to persist in humans for 20 times eight days (four to five months) despite choline therapy

Fig 12 represents a frozen section of the liver of a rat from this series which had been fed the choline supplemented diet for twelve days following the initial period of one year of choline deficiency A change is apparent in the structure of the persistent fatty cysts In the low power view (on the left), they appear blurred Higher magnification (on the right) reveals that the cells which form the wall of the cyst are beginning to be filled with small intracellular droplets of fat The appearance suggests to the observer that the lipid formerly contained within the lumen of the cyst is being reabsorbed into the cells of the cyst wall under the influence of the choline added to the basal diet The process might be compared to the absorption of fat from the intestinal lumen into the epithelial cells covering the villi When a higher magnification was prepared of a cyst in the liver of the same animal, fat was seen to be leaving the lumen of the cyst and entering the cells in the wall Higher magnification showed the abundant cytoplasm which these cells now possess, whereas when choline was absent from the diet, cells in the cyst walls were more often stretched and thinned



fills the cytoplasm of the surrounding cells in the form of small droplets of stainable fat

In the liver of another rat in this series which, after one year of choline deficiency, was fed a choline supplemented diet for 26 days before it was sacrificed, almost all the intra cellular fat which was absorbed from cysts and illustrated in Fig 13 was seen to have disappeared Only a few, very large fatty cysts still contained stainable lipid Eventually even these largest cysts begin to undergo regressive changes with passage of their contained lipid into the cells making up the cyst walls The process of fatty reabsorption by the cells in the walls of the cysts appears to occur more slowly in the larger cysts

Fig 14 is a representative field in a paraffin section of a liver showing the regressive changes in the larger cysts. The high-power inset shows that these fatty spaces are actually fatty cysts, as indicated by the multiple nuclei present in their walls. In interpreting the effects of lipotropic therapy in either experimental animals or man, it is thus demonstrated that it is important to distinguish between extracellular and intracellular lipid, for the former is mobilized much more slowly under the influence of dietary choline than the latter. When a representative field is examined from a frozen section of the liver of a rat fed the low-choline diet for one year, followed by the choline-supplemented diet for forty-eight days before it was sacrificed, only a few small droplets of fat, chiefly intracellular and probably originating from a few persistent large cysts, can be seen. These droplets are scattered and only an occasional large cyst persists. In the case of a rat fed the choline-supplemented diet for 62 days, after an initial period of one year of dietary choline deficiency, he fat in even the very rare, persisting fatty cyst is now eginning to leave the lumen and enter the cytoplasm of the rrounding cells

Fig. 15 is of a paraffin section from the liver of a rat fed the choline-supplemented diet for 63 days after the initial period of one year of choline deficiency. The few remaining scattered cysts can be easily seen in the low-power view. Under higher magnification (inset) the multicellular nature of the cyst-wall is apparent. All intracellular fatty vacuolation has almost completely disappeared except in the immediate neighbourhood of the cysts. Thus extracellular lipid has persisted in the liver of this animal for over two months despite treatment with lipotropic agents. This would correspond to several years in a human. Dietary choline has, however, been effective in mobilizing all the intracellular stainable lipid and much that is extracellular in these experimental animals. In co-operation with Professor E. A. Sellers, these studies are being continued for even more prolonged periods of observation to determine how long lipotropic

therapy is necessary to eliminate *all* the extracellular fat found in livers of rats fed diets low in choline for one year previously

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DISCUSSION FURTHER HISTOLOGICAL STUDIES OF FATTY INFILTRATION OF THE LIVER IN CHOLINE-DEFICIENT RATS

W S HARTROFT

I would like to discuss in somewhat more detail, the fate of fatty cysts in livers of rats maintained on low choline diets during the entire experimental period, in contrast with the type of experiment I reported first when the animals were given choline supplemented diets for varying periods after the preliminary feeding with choline-deficient food Once fatty cysts attain a diameter, in sections, approximating 100 micra, they begin to atrophy and are replaced by fibrous tissue This corresponds to the same period in which biochemical determinations of the total fat content of such livers have shown that the fat is leaving the liver as the fibrous tissue is deposited This is exactly what happens in the case of each cyst for as it atrophies it is replaced by fibrous tissue The next series of illustrations will demonstrate the pathways by which the fat leaves the cysts and the liver during the atrophy of fully matured cysts These photomicrographs have all been prepared from very thin frozen sections, using oil immersion lenses in most instances Two definite pathways for the escape of fat from fatty cysts have been found All figures are from tissues of Wistar rats fed diets low in choline for approximately one year

Fig 18 is an oil immersion photomicrograph of a fatty cyst in which only a rim of stainable fat is present Leaving the cyst at its lower right quadrant are a stream of small droplets of stainable fat They are entering the lumen of a bile canaliculus which communicates with the interior of the cyst by passing between two of the cells which form its wall Similar fields can be found in many places throughout sections of these fatty livers. The droplets may be followed in bile

canaliculi from the centrolobular *regions, where the* atrophic fatty cysts are present, to the portal regions Fat can also be demonstrated in the lumina of bile ducts India ink injected into the biliary systems of the *livers of choline* deficient rats can be found mixed with the fat in the bile ducts These findings are to be published in greater detail in the very near future

As the cyst walls are originally formed from parenchymal cells in the livers of choline-deficient rats, it is apparent that bile canaliculi may lie between many of these cells and that the lumina of the canaliculi are in communication with the interior of the cysts When the intracystic tension is sufficiently high, it appears that the lipid within the cyst can be forced out into the bile canaliculi Thus one pathway by which fat may leave the fatty cysts preceding their atrophy, is that consisting of the bile canaliculi and the bile ducts

Fig 17 demonstrates the *second pathway of escape for* cystic fat This consists of the *sinusoids and veins* A large fatty cyst is shown in the centre of the field Stainable fat (dark) is leaving the lumen of the cyst and entering that of a sinusoid which contains red blood cells (grey) Some of the red cells are leaving the sinusoid and entering the cyst replacing the departing lipid The process by which a communication between a cyst and a sinusoid is established is probably the same by which communications between cysts and bile canaliculi are opened The fate of red blood cells which enter ruptured fatty cysts is of interest but will not be discussed here (to be published elsewhere) The fate of *the stainable lipid droplets which enter the sinusoids will now* be discussed

When a large radicle of the hepatic vein was examined *in an area containing* fibrous tissue, which had replaced atrophic cysts a bile duct filled with ink (injected before death) was seen to lie to the right of the vein The vein was completely filled with stainable fat and red blood cells This demonstrates the manner in which the stainable fat reaches these vascular channels and is carried away from the liver



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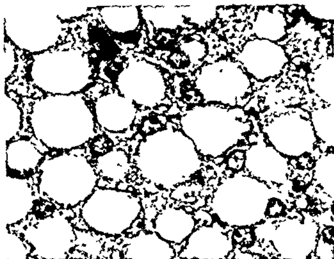
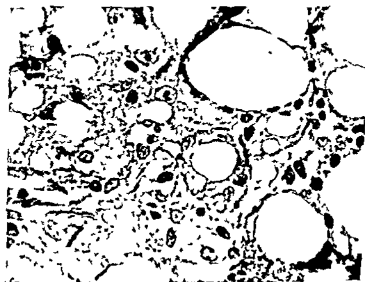


Fig. 49. Paraffin section of the liver of a rat fed a [illegible] diet for 10 days. Each parenchymal cell contains a single vacuole (previously filled with fat) in its cytoplasm. The diameters of these cells in the section measure from 18 to 28 micra. Stain and magnification as for Fig. 48 preceding.



Fig. [illegible]. Paraffin section [illegible] smaller spaces [illegible] diameter and the [illegible] These spaces are the [illegible] formed by the coalescence [illegible] which they previous[illegible]





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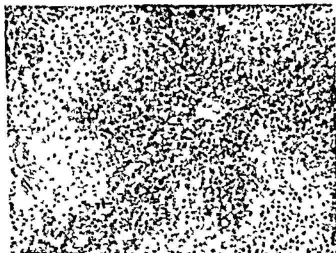
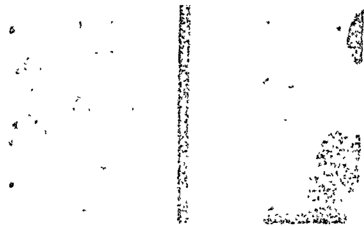
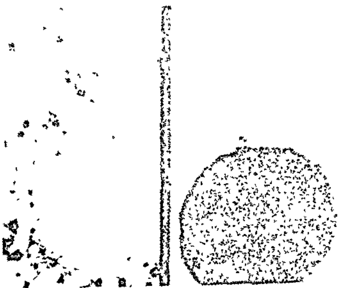


Fig. [illegible]. Frozen section of the liver of a rat fed the low-choline diet for seven days and then given a choline-supplemented diet for two days before it was sacrificed. The amount of stainable fat in this section is much less than that present in the section illustrated in the preceding figure. A central vein occupies the centre of the field. The periportal parenchyma is almost completely free of demonstrable fat and the small amount of lipid remaining is restricted to cells of the centrilobular region of the liver. (Oil Red O and Hematoxylin stain [illegible])





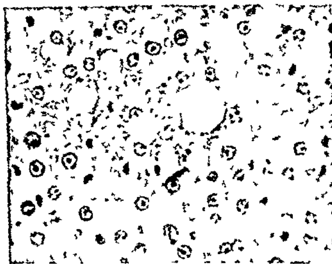
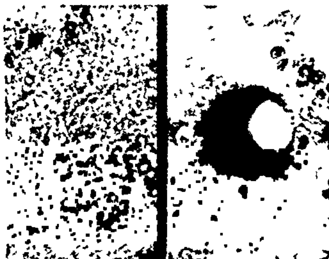


Fig. 11 [illegible] section of the l[illegible] of a rat fed the low-choline diet [illegible] one year and [illegible] supplemented diet [illegible] before it was weighed. The [illegible] these might be [illegible] taken for [illegible]



[illegible] 2. Frozen section of the l[illegible]er of a rat fed the low-choline [illegible]



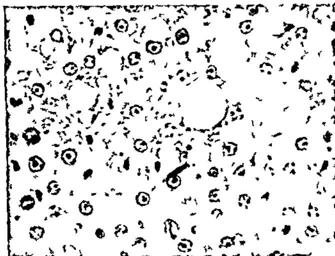


FIG 11 Paraffin section of the liver of a rat fed the low choline diet for one year and then given a choline supplemented diet for 8 days before it was sacrificed. The persisting fatty cysts are illustrated to demonstrate how these might be mistaken for intracellular fat vacuoles. Hematoxylin and eosin stain × 655

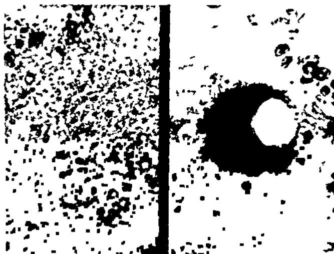


FIG 12 Frozen section of the liver of a rat fed the low choline diet for one year followed

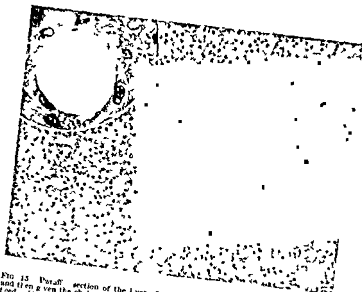


FIG 15 Paraffin section of the liver of a rat fed the low-choline diet for one year and then given the choline-supplemented diet for 63 days before the animal was sacrificed. This is the longest period of observation of this type of an animal which has been completed to date. Under the low magnification less than 10 fatty cysts can be shown. The multilocular nature of the wall of the cyst is shown in the high power inset. Hematoxylin and eosin stain ×65 and ×700. (Compare with Fig 14.)

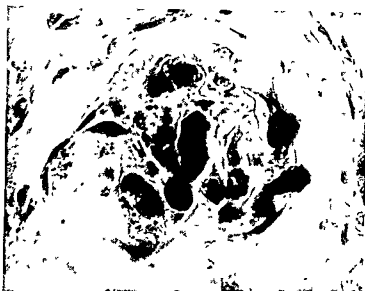


FIG 19 Glomerulus in a frozen section of the kidney of a rat fed a low choline diet for a period of almost one year The capillary loops are filled with masses of stainable fat (dark) which almost completely fill the vessel lumina *Oil Red O Hæmatoxylin* and Light Green stain × 655



An oil immersion photomicrograph of a frozen section of heart muscle taken from a rat fed the low-choline diet for over a year showed small droplets of stainable fat in the capillaries. The droplets had assumed an elongated form as they passed in the capillary between the cardiac muscle cells. Small focal areas of necrosis could be found in paraffin sections of the hearts of these animals and may be related to these small fat emboli. In frozen sections of the lung of these animals, droplets of stainable fat may be found in the small arterioles, even under low magnification. Under higher powers of the microscope fat droplets appear to be leaving the septal capillaries and entering the alveolar lumen where they are being engulfed by macrophages.

Fig 18, from a lung of a choline-deficient adult rat, shows large numbers of lipid filled macrophages. These macrophages have collected in large numbers in the lymphoid tissue surrounding the bronchioles. Many stainable fat droplets (inset) have filled the swollen cytoplasm of the macrophages and appear to be too large to pass through the lymphoid network. It is suggested that possibly these macrophages pick up the fat in the alveolar spaces which is carried to the lung from the liver by the blood stream. The lipid-swollen macrophages are drained to the peribronchial lymphoid tissue where they are caught. This would interfere with lymphatic drainage of the bronchi, thus predisposing the animal to bronchitis, bronchiectasis and bronchopneumonia, a disease syndrome to which rats are well known to be susceptible.

Fig 19 is a photomicrograph of a glomerulus in a frozen section of the kidney of one of these choline deficient rats. The glomerular loops are filled masses of stainable fat (dark in photograph). A higher magnification of one of the capillary loops of such glomerulus showed that the lumen of a capillary which had been cut crosswise was completely filled with stainable fat and some of the fat was seen to have left the lumen of the capillary and to have entered the capsular space.

Fig. 20 demonstrates the appearance of the medulla of such a kidney as seen in a frozen section stained for fat. Many plugs of stainable fat are blocking and entirely filling the lumina of the medullary tubules The high power inset shows that some of these lipid casts are becoming calcified It is possible that this tubular fat has its origin from lipid which has been brought to the kidney from the liver via the blood stream and that the fat has then entered the tubules and been precipitated in the form of casts in the medullary tubules where water reabsorption concentrates the glomerular filtrate

These studies appear to us to establish that rupture of fatty cysts into liver sinusoids of rats fed low choline diets for a year or more, results in a form of chronic, intermittent fat embolism. This might lead to focal cardiac necrosis, chronic bronchiectasis and bronchopneumonia, renal tubular obstruction and possibly other secondary effects

NEEDLE BIOPSY STUDIES OF THE LIVER IN CIRRHOSIS AND OTHER CONDITIONS

L SCHIFF

I WOULD like to show you some photomicrographs of specimens obtained by needle biopsy of the liver at the Cincinnati General Hospital Although they do not prove much of anything, I believe they are quite pertinent to this symposium Our experience with needle biopsy of the liver in cases of hepatic cirrhosis has been rather large We have biopsied over 80 patients with cirrhosis and have found a very high correlation with the histological changes as observed on necropsy One may miss the diagnosis in a few instances of post necrotic cirrhosis if the biopsy needle enters a nodule of regeneration

Fig 1 is a biopsy obtained from a white male patient of 64 years, a severe case of homologous serum hepatitis, on the 16th day of jaundice In Fig 2, after 39 days of icterus portal fibrosis is present in the biopsy specimen Experiences like these have been reported by Krarup (1941) and others

Hepatitis occurs in infectious mononucleosis, and recently a case of cirrhosis was reported following infectious mononucleosis (Leibowitz and Brody, 1950) When we did a biopsy on the liver of a patient with infectious mononucleosis we found the distention of the sinusoids with atypical lymphocytes, which has been described by Sherlock (1945) and Van Beek and Haex (1943)

A very interesting case was a man of 42, a severe diabetic whom we followed for 13 months He was jaundiced during this entire period, and the pattern of the laboratory tests was that of obstructive jaundice repeatedly negative cephalin flocculations, high serum alkaline phosphatase (49 Bodansky units compared to a normal level of 2–4 units), hypercholesterolæmia and hyperlipæmia

Our normal thymol turbidities are higher than those o Professor Maclagan since we use a barium sulphate standard but these high values were apparently due in part to the hyperlipæmia, as the zinc sulphate turbidity was negative He was quite jaundiced, with a maximum total serum bili rubin of 40 mg per cent Laparotomy performed after one month of jaundice revealed no extra hepatic obstruction We did several biopsies on this patient, and in all the predominant finding was that of biliary stasis In the biopsy obtained at operation, there was some evidence of hepatitis, but the prevailing opinion of a number of pathologists who viewed the biopsies was that the changes were essentially those of obstructive jaundice The biopsies were similar to those reported by Watson and Hoffbauer (1946) in their cases of so called cholangiolitic hepatitis. At post mortem the liver showed biliary stasis There was no cirrhosis I cite this as an example of a case of obstructive jaundice of 13 months' duration without development of any cirrhosis

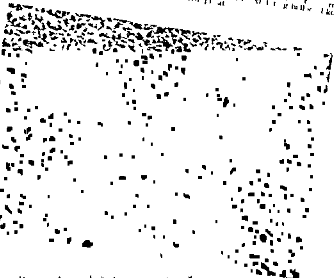
A liver biopsy obtained from a patient with sarcoidosis shows granulomas, as described by Scadding and Sherlock (1948), Van Beek and Haex (1943), and Van Buchem (1946) The patient was a young man with hepatosplenopathy and hilar and inguinal lymphadenopathy Fig 3 is a high power view of the biopsy, showing what is probably a pre Schaumann and a Schaumann body

Fig 4 was taken from a man of 52 with sarcoidosis Note the giant cells and the scattered granulomas Three months later the biopsy still showed many small scattered granulomas but no cirrhosis Ten months later there was definite fibrosis (Fig 5) We realize that this doesn't prove that sarcoidosis leads to cirrhosis, but we present it for what it is worth We were unable to find any fibrosis in the two previous biopsy specimens

Another interesting case was a coloured woman of 32 who presented a typical Banti's syndrome, with hæmatemesis, leucopenia, anæmia, splenomegaly, and œsophageal varices She had had a spleno renal anastomosis and a splenectomy



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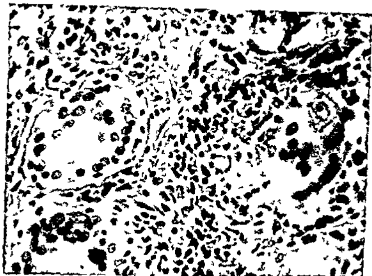


FIG 3 B W (C F 640) M l ary Cranulom A h gh power v ew demor strat ng a *pleomorphic non-epitheloid exudate and several of the giant cells containing vacuoles* and i clusions of nondescr pt character

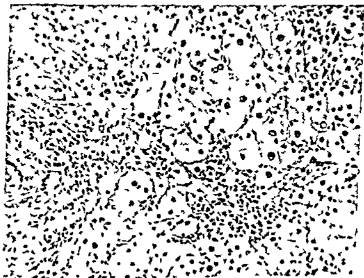


FIG 4 A I (C F 567) M l ary Cranuloma Lobular architectu l by
irregular strands of fibrous t ssue nfiltrated by l tes an l pl one
edge the margin of a tubercle conta n t cells n



FIG 5 A I (G E 608) Same case as Fig 4 10 months later Fibrosis is promine and there is not a well-defined micronodular structure Although inflammatory cel persist tubercles are no lo ger evident in th s spec men

1947 A liver biopsy at the time of operation was negative n May, 1950, she developed ascites Her serum albumin was 2 8 g per cent, there was 15 per cent retention of bromsulphalein at 45 minutes, the thymol turbidity was 7 units, and the zinc sulphate turbidity 29 A liver biopsy in May, 1950, showed granulomas consistent with sarcoidosis Is the sarcoidosis producing the clinical picture of cirrhosis? Does he have cirrhosis not revealed in the biopsy specimen? Is the ascites due in part to large glands pressing on the portal vein? We don't know the answers to these questions, but we feel nevertheless that this case is of great interest

We also studied a patient with a positive blood culture of *Brucella suis* Again at biopsy we found scattered granulomas The liver biopsy changes were similar to those in *Brucella abortus* infections, described by Spink and associates (1949) of the University of Minnesota

A similar case of a patient with a positive blood culture for *Brucella* has been studied by Drs Joseph Kirsner and William Ricketts of the University of Chicago Using a special Gomori Trichrome stain they found in the biopsy a considerable amount of liver damage with severe hepatitis Two and a half years later when the patient was entirely symptom free, liver biopsy showed typical granulomas with giant cells and also revealed the presence of fibrosis

A case of undefined granulomatous disease was that of a coloured male of 26, with chills and fever of eight months' ... ked anæmia slight leuco ... "liver profile" except for ... Bodansky units per 100 cc Liver biopsy showed the presence of scattered miliary granulomas of undetermined cause Various serological and blood tests were negative for any specific type of infection About a month later, granulomas were still present in the biopsy specimen Four months later granulomas were still present, but in addition, there was a questionable cirrhosis Dr Tracy Mallory has seen examples of granulomatous disease, otherwise undefined, progress into cirrhosis (personal

communication to the author) Dr Edward Gall believes that this patient may be developing cirrhosis, but, of course more time will be required to determine this

Granulomas and giant cells, indistinguishable from those seen in sarcoidosis, brucellosis, and even tuberculosis, were found in a biopsy taken from a woman with secondary syphilis

We have one case of fatty infiltration of the liver without development of cirrhosis The patient was a very fat woman, non alcoholic and non diabetic She has had seven liver biopsies during a period of over three years, and all of them showed the presence of fatty vacuolization After she had been on 4 g of choline a day with her diet unaltered, for six months, a biopsy showed that there was still a good deal of fatty vacuolization After four months of 16 g of choline daily, which she had some difficulty in taking, there seemed to be less fatty vacuolization, but I will admit that it's not valid to conclude that there was really less fat present After three years, she still has a good deal of fatty infiltration There is no evidence of cirrhosis You might say that we have missed the cirrhosis in our biopsies We believe, however, that since a nutritional type of cirrhosis is apt to be diffuse, it is not likely to be missed on needle biopsy of the liver So I show this as an example of fatty infiltration of the liver of at least three years standing without any cirrhosis developing

I know these observations prove very little, if anything, but if any of you have seen cases of sarcoidosis or of brucellosis develop cirrhosis, I should like to hear about them Spink and associates (1949) have listed some literature dealing with the occurrence of cirrhosis in brucellosis They point out that while a direct relationship between hepatic cirrhosis and brucellosis in the human has not been established, ' There is accumulating evidence that brucellosis may be a major accessory incitant in the genesis of severe and, at times, fatal cirrhosis "

I believe, therefore, that we should be on the look out for granulomatous processes as playing a role in some cases of cirrhosis of the liver

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C H BEST Dr Schiff s slides seemed to show that a lot of the intracellular fat had disappeared in his choline treated patients I suppose it is going to be necessary for the clinicians to go over their material—that was Dr Schiff s own suggestion—and see whether just the fat left within these large cysts remains, or whether there is intra cellular fat as well

W S HARTROFT I was very interested in Dr Schiff s slides, for they illustrated what appeared to me to be fatty cysts in livers of human alcoholics who had received dietetic treatment for several months This would appear to be the parallel in man of what I showed earlier in choline deficient rats fed diets with choline restored to them for two months

DISCUSSION ÆTIOLOGY OF CIRRHOSIS IN CEYLON

H A E KARUNARATNE

I wish to make a few remarks on the ætiology of cirrhosis in Ceylon and to show illustrations of the various histological types

It is well known that cirrhosis of the liver is widely prevalent in tropical countries It has been suggested that malnutrition plays an important part in its causation

There are seven conditions I should like to mention

1 *Cirrhosis of the Liver in Infants*

I would mention three cases of *infantile biliary cirrhosis* The children were aged one year, one year nine months, and two years and were all admitted for abdominal distension On examination the liver and spleen were enlarged, and there was jaundice and ascites They had been fed on cow's milk, been breast fed, or both There was no history of jaundice in the family of any of them Histological examination of the liver of one child showed atrophy, degeneration, and necrosis of the liver cells and it was noted that fibrosis did not originate in the portal tracts (Fig 1) In the second child a nodule of hyperplastic liver cells was seen

2 *Laennec's Cirrhosis in Children*

This condition is relatively common in Ceylon Within a period of six months I saw five typical examples in children whose ages were three and a half years, four years, six years, and seven years (two) All these cases showed marked cellular infiltration of the portal tracts, and in three there were, in addition, definite bands of fibrosis uniting the cell infiltrated portal areas, thus conforming to the "inter insular" stage of Fiessinger A certain degree of fatty change was present in all of them One case (Fig 2) was of special interest as showing not only the completed picture of portal cirrhosis, but also

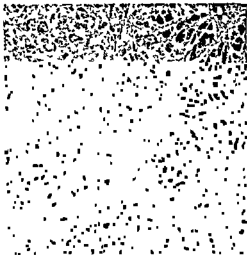


FIG 1 Section of liver from child with biliary cirrhosis

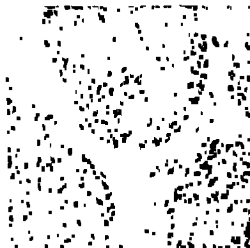
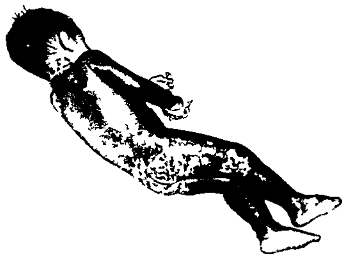


FIG 2 Laennec's cirrhosis in a child with hookworm infestation



F G 3 Kwa h orko Sk n les ons in a ch ld

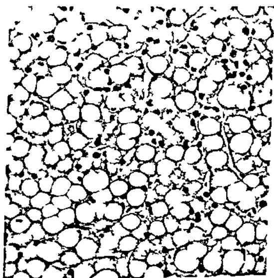


FIG 4 Fatty infiltrat on of l er in infant e kwash orkor

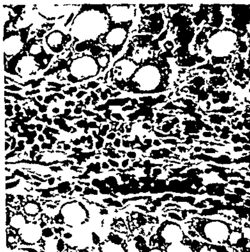


Fig. 5 Cellular infiltration of portal tracts in infantile kwashiorkor

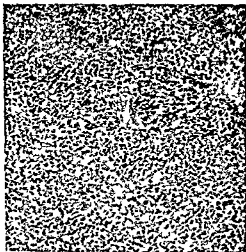


Fig. 6 Early cirrhosis and fatty infiltration in adult kwashiorkor

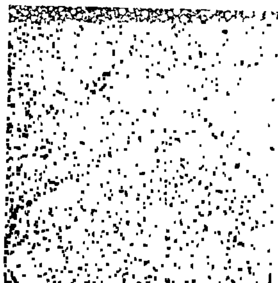


Fig 7 Fatty infiltration and early fibrosis of the liver from a case of ulceration of large intestine



Fig 8 Post necrotic fibrosis of liver after infective hepatitis

because it was one where clinical evidence of the condition was ascertainable during life The child was emaciated, with puffiness of the face and swelling of the feet. The spleen and liver were both enlarged. There was ascites There was no jaundice Death occurred after a severe hæmorrhage per rectum Post-mortem examination showed a markedly cirrhotic liver The veins at the lower end of the œsophagus were dilated and varicose. The mucous membrane of the stomach and intestine was markedly congested

As all these cases were infected with hook-worm and no other recognizable cause was present in any of them, it is very likely that the worm infestation was an important, or perhaps the main ætiological agent in the production of the cirrhotic condition. A secondary or conditioned malnutrition resulting from the bowel condition cannot, however, be excluded It may be mentioned that Vint found cirrhosis of the liver quite common in East Africans in association with intestinal helminthiasis He believed that toxic substances produced by intestinal helminths was the causative factor

3 *Kwashiorkor (Infantile Pellagra, Malignant Malnutrition, Fatty Liver Disease in Infants)*

This is a brief summary of 14 cases aged from four months to three years In 11 there was enlargement of the liver, œdema, and wasting In two there was œdema and wasting, but no enlargement of the liver In one there was enlargement of the liver and wasting, but no œdema Lesions of the skin, mucous membranes and muco cutaneous junctions were present in most of them (Fig 3) Few showed "crazy pavement" dermatosis, many showed mosaic-skin, angular stomatitis, hyper-pigmented patches and areas of depigmentation Two of the cases showed excoriation around the anus In most of the babies the hair was thin and scanty Some showed depigmentation of the hair Many of them suffered from diarrhœa One of the cases was a little girl aged one year seven months who had enlargement of the liver, wasting, thin, scanty and depigmented hair, and hyper-pigmentation of the skin in the region of the right knee She had been

breast fed for one year and thereafter had been fed on rice A biopsy of her liver showed extreme fatty infiltration (Fig 4), every cell was distended by a large globule of fat Fig 5 shows cell infiltration of the portal tracts, in high power, in a second case

4 *Adult Kwashiorkor*

I would just mention one case in a patient aged 50 who had generalized œdema, ascites, loss of hair on the scalp and scanty hair in the axillary and pubic regions He had hyper keratosis of the skin and pigmented areas Plasma protein was low, the albumin being reduced and the globulin increased There was also keratomalacia pointing to vitamin A deficiency Biopsy of the liver showed early cirrhosis with some fatty infiltration (Fig 6)

5 *Ulceration of the Large Intestine*

Biopsies of the liver in cases of amœbic ulceration and bacillary dysentery have shown marked fatty infiltration with early fibrosis (Fig 7)

6 *Alcohol*

In Ceylon alcohol is not a frequent cause of cirrhosis Professor Fernando reported on 87 cases of Laennec s cirrhosis and noted a history of alcoholism in less than one third of the cases, and in some of these there was definite evidence also of dietetic deficiency

7 *Post-necrotic Fibrosis*

Professor Fernando also examined 15 cases of post necrotic fibrosis following infective hepatitis, and found a definite history of dietetic deficiency in six of them Fig 8 is taken from a typical case of post necrotic fibrosis

I am grateful to Professor Fernando and to Professor de Silva for their assistance and for the use of their records

'SSION THE ÆTIOLOGY OF HEPATIC CIRRHOSIS

M BJORNEBOE

The epidemic of hepatitis in Denmark in the forties gave us a special opportunity to study the relation between hepatitis and chronic disease of the liver The typical chronic lesion of the liver in this epidemic has been subchronic atrophy of the liver but also typical portal cirrhosis has been found

To find the exact relation between acute and chronic cases Ryssing from our department in 1946 registered all cases of acute hepatitis in Copenhagen observed during six months He did this work by personal interviews with the practitioners in Copenhagen and their jaundiced patients All cases were re-examined six to twelve months later During this time 1 140 cases of hepatitis were found Of these cases 31 (2 9 per cent) were severe Seventeen died of subchronic atrophy and 14 became chronic All these 31 cases were above 40 years of age (26 above 50) and all except two were women They occurred among 242 cases of hepatitis who were older than 40 years that is in 12 8 per cent of this age group These figures are certainly minimum figures since a longer observation time probably would have disclosed more cases of chronic hepatitis This little part of the Danish hepatitis epidemic described by Ryssing demonstrates the fact observed by several authors early in the epidemic that the prognosis is worse for women and especially for women past the climacteric age

We have together with Raaschou studied the pathological aspect of the disease by studying all cases of portal cirrhosis and subchronic atrophy of the liver at our hospital autopsied from 1928 1947 It is evident from Fig 1 that the number

of cases of subchronic atrophy of the liver was very small before 1944 Since 1944 an increasing number of cases of this rare disease has been observed and practically all of them were seen in women The case records for these patients all showed a history typical of chronic hepatitis The number of cases of portal cirrhosis during these 20 years is fairly constant and does not increase during the hepatitis epidemic in

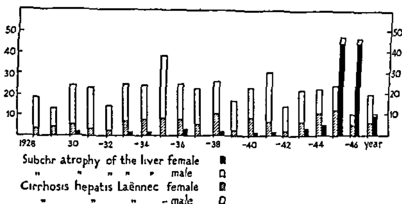


FIG 1 The occurrence of subchronic atrophy of the liver and cirrhosis hepatis Laennec in post mortem material of the Kommunehospital from the years 1928–1947
(M Bjørneboe and F Raaschou *Acta med Scand* supplementum 234 41–62, 1949)

the forties The only change is perhaps a relatively greater proportion of women among cases of portal cirrhosis By going through the case histories for the 33 women with portal cirrhosis observed from 1944–1948 it was evident that nine had had a typical chronic hepatitis The average duration of symptoms was not longer for these cases of female portal cirrhosis than for the average of cases with subchronic atrophy of the liver

There is abundant evidence in this material from the Danish epidemic that portal cirrhosis and subchronic atrophy of the liver may be caused by hepatitis

We have tried to find out why hepatitis may take a severe course In the first place there is no reason to believe that a new type of hepatitis virus has been the cause of the severe cases Several authors have been able to show that acute hepatitis immunizes against chronic hepatitis Very few cases of chronic hepatitis in this epidemic have had hepatitis before

It is evident that hepatitis in a woman past the climacteric age in this epidemic has a bad prognosis This is in agreement with earlier publications Ryssing has been able to collect 80 cases of subchronic atrophy of the liver from the literature Twenty three of these were women and half of these women were over 40 years

The question is now why so many middle aged and old women got hepatitis in Denmark during this epidemic We have no complete explanation for this fact but it has been shown that older age groups as a whole have been more frequently attacked this time than in earlier epidemics (Ryssing) Perhaps the explanation is that this epidemic was much greater than any observed before in Denmark It has been shown in other epidemic diseases that the incidence among older age groups is relatively higher the greater the epidemic is

Why older women have a more unfavourable prognosis than younger is still a problem Several explanations are possible In the first place Gyorgy has shown that œstrogenic hormones have a liver protecting effect in animal experiments In this connection it might be of interest that of 13 consecutive cases of chronic hepatitis in women below the menopausal age observed in our department, seven had some gynæcological complication Another factor of importance may be constipation Munthe Fog from our department observed during an investigation of diet habits and drug intake of women with chronic hepatitis that there was a significant higher consumption of laxatives in this group than in a control group Gyorgy has suggested after hearing about this observation that constipation and resorption of

bacterial toxins may play a role in the course of the disease. This would be a parallel to the very interesting observation made by *György* of the importance of bacterial flora for dietetic liver damage in rats.

GENERAL DISCUSSION

C J WATSON I was particularly interested in some of the points brought out in Dr Hartroft's presentation I have had the privilege

portal in origin Dr Davies, who has been studying Kwashiorkor in Uganda, informs me* that he believes the fibrosis in that *condition* is initially portal rather than central This raises the question whether fatty cirrhosis in the animal is entirely identical with that in human beings, and to what extent we can draw analogies We have been *interested in this question from the standpoint of pigment metabolism* It may be pertinent that dogs and rats may be literally killed with alcohol either in acute or chronic experiments, with development of fatty liver, but without development of cirrhosis In neither species

being the type which Dr Bjorneboe has

cirrhosis, I think it is at least interesting that alcohol does not cause a disturbance of porphyrin metabolism in dogs and rats but does in

of cirrhosis as it occurs in the U S,

cal cirrhoses.

are two main

tty cirrhosis,

*Personal communication

need not be fatty by the time the individual comes to autopsy because

time

[illegible] I choose to

degrees of transition between [illegible] on the [illegible], [illegible] toxic nodular, or so-called healed acute yellow atrophy, on the other

L. S. P. DAVIDSON Few experienced physicians feel confident in

to hepatic diseases is of no importance in prognosis I know of several families in which more than one member died of hepatitis associated with alcoholism Although the patients had been heavy drinkers they drank no more than many individuals who never develop chronic hepatitis Moreover, belonging, as they did, to the higher economic income groups, I do not believe that the hepatitis could have developed simply from dietary deficiency Hence I believe that they were born with inferior livers, so I suggest that *age, toxic agents such as alcohol,* infection, dietary deficiency and hereditary inferiority of the liver are all factors which must be taken into account when we are considering the prognosis of hepatitis

J STOKES *In connection with these remarks and those* Dr Bjørneboe has made it is of interest that children appear to develop a permanent immunity to the virus of infectious hepatitis, if they are exposed to it at a very early age I agree that cirrhosis is more apt to [illegible] later in life Possibly [illegible]

[illegible] nurses had been exposed to floors where children were suffering from a mild gastro intestinal disease which was being kept alive by the continuous addition of susceptibles Also a few chronic active cases appeared to carry the virus in their stools This group of children showed positive liver function tests, and there had been two cases among the children of hepatitis with jaundice It was prevented temporarily by the use of gamma globulin, *but when gamma globulin* [illegible] the nurses The nurses had a much more [illegible] usually their jaundice [illegible] of hepatitis in another [illegible] or the epidemic virus, [illegible] *id among the younger* individuals was the same, but *the older individuals had a much longer* [illegible] were much more severely affected by the disease

[illegible]

means of the natural route for the protection of [illegible] cirrhosis in later life

H W KOSTERLITZ The observations of Dr Bjørneboe, Dr Davidson and Dr Stokes have encouraged me to present some findings which may possibly be relevant

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F O MACCALLUM Could our American colleagues tell us whether now eight years after the great episode of the yellow fever vaccine jaundice there is any evidence of an increased rate of cirrhosis in the States? How long does it take to produce cirrhosis? How quickly can you get it? And have you any evidence of how many cases who had these icterogenic batches and who apparently did not develop jaundice are turning up with sub chronic hepatitis or cirrhosis? An example of this problem came to notice a few days ago A Serviceman who had been injected with an icterogenic batch of yellow fever vaccine in November 1943, and who had then shown no symptoms of any liver damage, suddenly developed jaundice and cirrhosis in 1949 and died within a

jaundice at the time subsequently died of hepatitis much later Aren't these figures very difficult to correlate ?

C J WATSON They're very hard to obtain , it's extremely difficult to determine the incidence of cirrhosis in the vast number of individuals [illegible]

[illegible]

there were 32 that had a previous history of jaundice, and of these [illegible] had a history that was at least *strongly indicative of infectious hepatitis* [illegible] the same year of [illegible] nothing [illegible] ere were

[illegible]

[illegible] m [illegible] re [illegible] In [illegible] er [illegible] rs

ago, the figure was around 70 per cent (Die Leberkra [illegible] in, Julius Springer 1937) If one is dealing with preponderantly alcoholic [illegible] likely to be masked and [illegible]

J W McNEE I first began to see infective hepatitis [illegible] form about 1925 I watched a series of small outbreaks in Preparatory

of cases of the acute disease go on to the *chronic* sequel, but these patients, I think, may well account for a number of the cases of cirrhosis seen in Britain in which alcohol as a causal factor can be excluded

[illegible] somewhat of the age group, the incidence of chronic disease being, as Dr. McNee said, extremely small

P. CAZAL. In the South of France both epidemic hepatitis and

appears when the specific infection disappears, at other times the fibrosis remains after the disappearance of the inflammatory lesions and may increase later These inflammatory cirrhoses may appear even in children *Other inflammatory causes of cirrhosis are tuberculosis severe* malaria and congenital syphilis The cirrhosis has been confirmed by *histological and clinical* examination It may develop without *any fatty* infiltration or centrilobular necrosis In our studies at Montpellier we found that more than 40 per cent of all cirrhosis cases were inflammatory, the others being of alcoholic, obstructive or other nature I have also found some cases of cirrhosis in children whose mothers were Rh *negative and immunized by the Rh antigen of the father*

F WUHRMANN In Switzerland we find that at least 70 per cent of *our cases of liver* cirrhosis are caused by chronic alcoholic intoxication the rest are of posthepatitic or unknown origin (the so called cardiac cirrhosis not included) In our country every year between 800 000 000 and 900 000 000 *hard Swiss francs are spent* on alcoholic beverages This amount is drunk by a relatively small percentage of the people and that is why you can see such an impressive number of cases of liver cirrhosis caused by alcohol

... depends very much on the

... who drink almost 10–15 litres
... an very

... which is
... ry man
who drinks enormous quantities of alcohol will get cirrhosis of the liver, and I think there is much truth in the old rule of the French clinicians who more than a hundred years ago used to say, "*Ne devient pas cirrhotic qui veut*

C H BEST Regarding alcohol I don't think it's legitimate to say that alcohol is toxic to the *liver as long as an equicaloric* amount of ... fatty infiltration and

A GAJDOS Research on nutritional cirrhosis ... *alcohol to a less important position as an aetiological factor in human* cirrhosis and several authors consider liver cirrhosis to be a result of a lack of methionine or choline in the diet

nutritional conditions, and the frequency was generally attributed to a high alcohol consumption The nutritional conditions were totally transformed by the German occupation, and the consumption of foods

of the diminution of alcoholic consumption.

Since the war, the number of patients with alcoholic cirrhosis has increased, and in the last two years we have observed about five times as many cirrhotic patients as during the German occupation At the University of Lyons the situation is the same and Delore and co-workers show that the number of cirrhotic patients is actually 4 per cent of the total number of patients admitted, against 1 per cent during the years 1940 1946

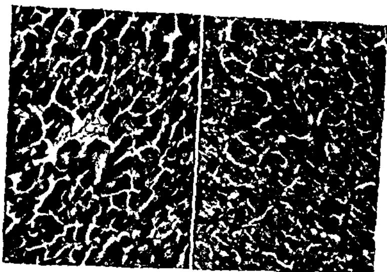
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enough

DISCUSSION DEVELOPMENT OF TRABECULÆ IN CIRRHOSIS, AND PANCREATITIS DUE TO PROTEIN DEFICIENCY

H POPPER

Encouraged by Dr Watson's question as to the origin o the connective tissue trabeculæ in human portal cirrhosi I would like to refer to some studies which are being carrie out by Drs Elias, Petty and myself Convincing evidenc exists that in cirrhosis produced in experimental animal by carbon tetrachloride (Ashburn *et al*, 1947) and nutritiona deficiencies (Hartroft 1950, Glynn *et al*, 1948) the connec tive tissue trabeculæ originate in the centre and the trabecul contain vessels which can be injected from the hepatic vein Study of human material gave us the impression that a least some of the trabeculæ in portal cirrhosis should be o portal origin Therefore, injection masses of contrastin colours were injected into the portal and hepatic veins o nine human livers with cirrhosis Histological study of these livers shows that the central part of the nodules and lobules is injected from the hepatic vein whereas their periphery is injected from the portal vein, the vessels here being much smaller The connective tissue trabeculæ, however, reveal a dense, vascular network injected from both the portal and hepatic veins, as can be seen from the difference in colours In addition, in many places communications of relatively large branches of the portal with those of the hepatic vein can be seen in the trabeculæ This indicates characteristic differences between the experimental cirrhosis in rats, which is primarily central in origin, and human cirrhosis, in which the trabeculæ contain vessels of portal and hepatic origin and which possibly, therefore, are derived from both central and portal spaces



A B



C

FIG 1 Photomicrographs of hæmatoxylin eosin sections of rat pancreas (× 285)

A Control animal (after 24 hours fasting) The cytoplasmic basophilia on the base of the acini is marked.

B Twenty four hours after ethionine … injection … cells are … and … zymogen … basophilia …

C … ethionine … structure … cells …

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I should like also to present a set of experiments which are not primarily connected with the liver but which were stimulated by the work of Davies (1948) and Trowell (1950) on Kwashiorkor. In this condition they have demonstrated pancreatic changes which were considered the result of protein deficiency. The pancreas, which forms the greatest amount of protein per unit weight in the body, is stimulated by a high carbohydrate diet and is unable to form sufficient protein to satisfy the demand. Dr Farber and I have been studying the fatty liver which develops after administration of ethionine to fasted female rats. This substance has the same formula as methionine, except that an ethyl group replaces the methyl group and is therefore considered an antagonist of methionine. It is assumed that by its administration the methionine utilization is completely interrupted within a few hours and strong evidence has been presented that the protein synthesis is inhibited (Simpson *et al.*, 1950). Within twenty four hours after the administration of 1 mg ethionine per gram body weight, the pancreas appears grossly large and swollen. Histologically the basophilia of the acinar cells has almost completely disappeared whereas otherwise the cells appear still perfectly intact (Fig. 1A and B). Forty eight hours after administration of ethionine, necrosis of the acinar cells is noted. The acinar structure is disturbed and interstitial infiltration with histiocytic cells and leucocytes is noted (Fig. 1C). After longer intervals fat necroses appear. The lesion is completely prevented by simultaneous administration of methionine. It does not only occur in the presence of a fatty liver. In male animals, in contrast to females methionine fails to produce a fatty liver, probably due to a protecting effect of testosterone which reduces protein requirements (Farber *et al.* 1951). However, the pancreatic changes are identical in males and females. Dr Farber has also shown that administration of glucose prevents the hepatic changes but it does not influence the pancreatic lesion. Choline prevents neither the fatty liver due to ethionine nor the pancreatic changes. We would like

to interpret this experimental evidence as a strong support for the assumption that the pancreas degenerates in the presence of severe protein deficiency. We suspect that this may be an ætiological factor in at least some forms of human chronic as well as acute pancreatitis.

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GENERAL DISCUSSION

G. R. Cameron: A naive attitude is assumed by certain experimentalists in [illegible] liver. It ha[illegible] other people [illegible] ... to show up a landmark. Now I think [illegible] etry [illegible] uch [illegible]

appears at the centre of the lobules and also at the periphery. We stressed the active nature of the growth of peripheral fibrosis. We liked very much the generalizations of Fiessinger and his classification of different stages of cirrhosis.

In the more recent experimental work it is assumed that in a liver which is changing the landmarks quoted above are fixed. They are nothing of the sort. They change. Once cirrhosis is started off the lobules may collapse and the landmarks can be completely altered. *For instance, central veins can be brought right up against the periphery of lobules.* In this way the most bizarre effects may be produced and the whole of the geometry goes wrong. I don't believe that the older work on the distribution of *fibrosis is vitiated by the supposed accuracy* of the "landmark" methods.

PART III

ÆTIOLOGY OF PORTAL HYPERTENSION AND ASCITES, AND ITS TREATMENT

Chairman: C. H. BEST

THE RELATION OF CERTAIN MECHANICAL FACTORS TO THE PRODUCTION OF ASCITES

W. VOLWILER

In discussing the problem of the fluid retention which occurs in the patient with liver disease, one can divide the factors responsible into two general groups: we have first those factors which lead to the general retention of fluid throughout the body, and secondly those mechanical factors which cause the localization of the œdema fluid in the peritoneal cavity. It is with certain of this second group of mechanical factors that I am primarily concerned.

Now, just what is the exact relationship of portal vein pressure to the formation of ascites? I propose to demonstrate to you that chronic portal hypertension may exist without there being ascites, and, on the other hand, that ascites without peripheral œdema may be found in the absence of an elevation of the portal vein pressure. I shall then consider briefly the altered lymphatic circulation of the liver as a possible ætiological factor in the production of the ascites associated with severe venous congestion of the liver.

Human patients having congestive splenomegaly and œsophageal varices caused by extra-hepatic block of the portal vein do not ordinarily have ascites. In such cases, pressures taken at operation in branches of the portal vein have been published as follows. Rousselot (1940) found portal pressures

in congestive splenomegaly without ascites of 33, 37, 40 and 46 5 cm of water, Blakemore (1947) found an average of 33 cm of water in six cases, and Linton (1948) had pressures of 23, 34, 42 and 49 cm of water in similar cases

Of course, it would be very nice for my argument if I could assume that the portal vein pressure for normal man is the same as for all lower mammals Certainly the rat, the cat, the dog and the monkey all have normal portal vein pressures of less than 12 cm of water *It is important to* realize, however, that there exists an extreme lack of published figures for normal man, in the few reports existing figures up to 22 cm of water are recorded (Bellis 1942) I hope that Mr MacPherson will have further data on this point

Next, there are records of markedly elevated portal pressures in patients with cirrhosis and œsophageal varices who did not have ascites In Pattison's series for instance, the patients who did not have ascites were found to have portal pressures as high or higher (33, 35 40, 60 cm water) than those who had ascites (12, 25, 28, 32, 35, 35 cm water)

There is also good evidence on this point in the dog At least three types of preparations have been made in which moderate to marked elevation of the portal vein pressure has occurred without the formation of ascitic fluid

First, there is the production of silica fibrosis of the liver by serial intra portal injection of ground quartz A very marked collateral circulation to the intrahepatically obstructed portal circuit develops, I believe this is the only experimental method which has sometimes resulted in the formation of true submucosal œsophageal varices in the dog After the first eighteen months of the silica fibrosis, Rousselot and Thompson (1939) found elevated portal venous pressures (22 5 23, 26, 29 5 cm water) but at this stage, no ascites (Rousselot, personal communication)

Dr Grindlay, Dr Bollman and I have repeated these experiments, in modified form, and confirm these findings In the peritoneal cavities of our dogs fifteen months after the first intra portal injection of ground quartz, we see marked

hepatic scarring and a very impressive collateral circulation to the obstructed portal vein exhibited mainly by the numerous dilated and tortuous veins connecting in the base of the mesentery with the inferior vena caval circuit. Yet in spite of this impressive collateral formation there is often no ascitic fluid.

The second dog preparation demonstrating this point is the progressive constriction from cellophane bands placed simultaneously around the abdominal inferior vena cava and main portal vein. An extensive collateral circulation develops. Some of these dogs are found to have elevations of their portal vein pressure up to 27 cm. of water but without the spontaneous formation of ascites (Volwiler, Grindlay and Bollman, 1950).

The third dog preparation is the direct aorta to portal vein arterio venous fistula accomplished through vein graft by Cohn and Parsons (1950). Portal vein pressures measured two weeks after the formation of the fistula were 30, 34 and 39 cm. of water. No ascites was found (Cohn, personal communication).

Now let us turn to the next portion of the problem. Can oedema fluid form and be localized to the peritoneal cavity in the presence of a portal vein pressure within the range of normal? I know of no supporting data in the human as yet. Dr. Grindlay, Dr. Bollman and I designed some dog experiments to test this point. We found we could produce in this experimental animal a marked ascites without peripheral oedema by either of two methods (Volwiler, Bollman and Grindlay, 1950). The first was to constrict through the application of a cellophane band the thoracic inferior vena cava so as to cause marked venous congestion of the liver. This method for producing ascites I shall refer to as Preparation 1. Our second method was to render dogs with obstructed portal veins hypoproteinæmic through plasmapheresis; these animals are classified as Preparations 2 and 3 on Fig. 1. This shows the average portal pressure in each of our dogs who were made to have ascites by either of the two methods.

These measurements were taken in the unanæsthetized trained dog in the normal standing position by an ingenious method originally devised by Dr Hoffbauer (1948)

Under Preparation 3 it is noted that one cannot expect to produce chronic portal hypertension in the dog by simple ligation in two stages of the main portal vein If as in Preparation 2 one constricts also the abdominal inferior vena cava some of the animals will have moderate elevations of their portal pressure some of this group also had normal portal pressures None of the dogs of Preparations 2 and 3 spontaneously formed ascites However when they were made hypoproteinæmic through plasmapheresis an ascites without peripheral œdema formed This ascitic fluid was very thin and watery and had a protein content of about 10 per cent that of the plasma

We believe that the thin ascites in these animals is due to an escape of fluid from the capillaries as a result of lowered plasma osmotic pressure We have supposed that the localization of the œdema fluid to the peritoneal cavity here is caused by the presence of an increased filtering surface of the expanded splanchnic capillary bed which is relatively unopposed by tissue tension

All dogs of Preparation 1 with the severely congested livers spontaneously developed a marked ascites From Fig 1 it is noted that a few of them had portal pressures at the upper limits of the normal range when determined within one week of the formation of ascitic fluid Those with the highest pressures had no greater collection of ascitic fluid and seemed to form ascites no faster than those with the lowest

I would like to conclude from these dog experiments that œdema fluid may be localized to the peritoneal cavity when the portal vein pressure is within normal limits

Now if the ascites associated with severe venous congestion of the liver is not caused primarily by portal hypertension what is the mechanism of its formation? We believe it represents an overflow of liver lymph (Volwiler Grindlay and Bollman 1950) You may recall that this is the same

conclusion reached by the English physiologist, Charles Bolton, 20 years ago (Bolton and Barnard, 1928, Bolton and Barnard, 1931)

Liver lymph flow increases with the first signs of venous congestion of that organ. The hilar liver lymphatics of the dogs with congested livers become hugely dilated as they

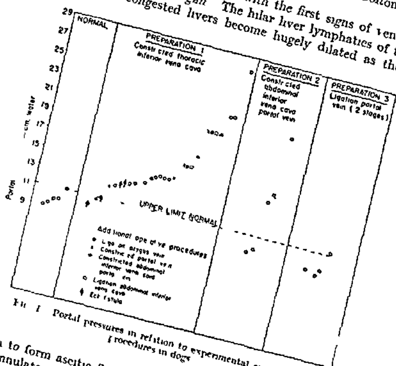


FIG. I. Portal pressures in relation to experimental operative procedures in dogs

begin to form ascitic fluid. Dr Grindlay was actually able to cannulate the main efferent liver lymphatic with plastic tubing (Grindlay *et al*, 1950) so that we could quantitatively collect its lymph and measure the rate of flow. As anticipated, this amounted to 10 to 20 times the normal flow, or, for a 12 kilogram dog roughly 5,000 cc per twenty four hours as contrasted to the normal figure of 250 cc. Not only are the

hilar liver lymphatics engorged, but so also are the numerous small subcapsular lymphatics (Fig 2), previously called attention to by Bolton and Barnard (1931) and by McKee *et al* (1949), these are often present over the entire surface of the liver

The ascitic fluid of dogs of Preparation 1 has a remarkably high protein content—about 60 per cent of the plasma level, and has been shown to have an electrophoretic pattern very similar to that of the plasma (McKee *et al*, 1949) Liver lymph is known to have a very high protein content—about 80 per cent of the plasma concentration Obviously, then, hepatic lymph would be an excellent source for the ascitic fluid associated with severe venous congestion of the liver

This hypothesis that the engorged liver forms ascites primarily by pouring lymph into the free peritoneal cavity, for final proof requires the demonstration of actual flow of fluid and other plasma constituents across the lymphatic walls into the free peritoneal cavity The circulation of this type of ascitic fluid, and its rapid equilibrium with the plasma have been amply demonstrated (McKee *et al*, 1948, 1949, 1950, Volwiler *et al*, 1950) but I must admit that the major site of fluid inflow has not yet been actually demonstrated beyond possible doubt

To reiterate although portal hypertension is no doubt of great importance in urging localization of œdema fluid intraperitoneally, it may exist chronically without there being ascites When ascites does exist, other mechanical factors such as the engorgement of liver lymphatics may, in certain situations, be of greater importance in its formation

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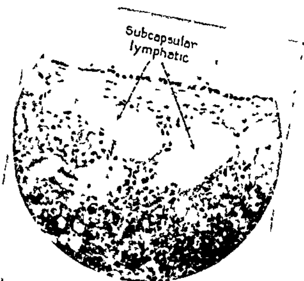


Fig. 2. Subcapsular lymphatics of liver engorged after experimentally produced venous congestion of the organ

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FACTORS IN THE MECHANISM OF ASCITES

H G KUNKEL

It is difficult to evaluate the relative role of a single factor such as increased portal pressure in the mechanism of ascites because of the numerous different agents, known and un known, that are involved It has become apparent however, that different patients with cirrhosis vary greatly in both the number and relative importance of causal agents inducing ascites At one extreme is the middle aged alcoholic who is thin and dehydrated and has accumulated ascites steadily for a considerable period of time He represents the most intricate picture where, in addition to increased portal pressure and hypoalbuminæmia, extreme renal retention of sodium and various antidiuretic materials undoubtedly play a role Such a patient rarely responds to the elimination of a single factor by various forms of therapy Elevation of the serum albumin level to normal by means of intravenous albumin injections only occasionally produces a significant diuresis

At the other extreme is the young, fairly well nourished individual with early ascites and severe hypoproteinæmia, approaching that seen in the patient with nephrosis The distribution of fluid is only slightly different from that in the patient with nephrosis, varying only in a greater tendency for the fluid to appear in the abdomen Such a picture is usually seen in patients with the post necrotic type of cirrho sis and in certain young girls with liver disease of unknown ætiology Despite the accumulation of fluid, a significant amount of sodium is always found in the urine A search for antidiuretic materials, tested both in the rat and dog, has not revealed such definite effects as in certain patients with nutritional cirrhosis and long standing ascites The hypoal buminæmia and increased portal pressure here appear to

be dominant in the mechanism of ascites Such patients tend to respond readily to intravenous albumin injections with a diuresis and loss of ascites A study of this type of

Fig 1 illustrates the three-year course of a seventeen-year-old girl with post necrotic cirrhosis following infectious

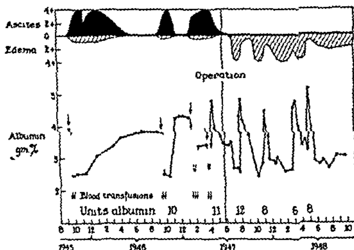


FIG 1 Relation of the serum albumin concentration to the formation of ascites and œdema in a patient with cirrhosis of the liver before and after a spleno-renal anastomosis

hepatitis Serum albumin levels throughout this time are plotted They were all carried out at the same time on sera that had been stored in the cold The Howe method, modified to achieve an accuracy of ±3 per cent, was used The upper portion of the chart indicates the approximate ascites and œdema that were present The large arrows over the albumin curve signify œsophageal hæmorrhages The points

where albumin was given are indicated by numbers of 25 gram units at the bottom of the chart

The patient suffered the first œsophageal hæmorrhage at a time when she was well nourished and was free from ascites and œdema Despite two blood transfusions the albumin level dropped severely and ascites gradually formed, requiring a paracentesis Gradually, while on no specific therapy, the albumin level rose to approximately normal over a period of almost five months During this time the ascites gradually disappeared Approximately one year later the patient again had a severe hæmorrhage, followed by a very low albumin level and a return of ascites She fell into a coma for two days from which she gradually recovered This time, 10 units of concentrated human albumin were administered intravenously with an immediate elevation of the serum albumin level and a diuresis with loss of all ascites The patient was able to preserve the elevated albumin level for a period of four months, when a third hæmorrhage developed, again resulting in coma and ascites Albumin was not given and the ascites persisted for several months along with a subnormal serum albumin level A fourth hæmorrhage then developed, resulting in the third episode of coma, that was treated with blood transfusions with very slow improvement A diuresis of the ascitic fluid was obtained following the administration of 11 units of concentrated human albumin This time, however, she was unable to preserve the artificially elevated serum albumin levels because of a gradual deterioration in her general condition A spleno renal anastomosis was carried out by Dr Alfred Blakemore At operation the portal pressure was noted to be just over 300 mm of water, which then fell to 110 mm of water following the establishment of the shunt Following operation there were no further hæmorrhages (to December, 1950), and no further ascites developed However, for some reason, not clearly understood, the serum albumin level fell to a low range and considerable œdema developed in the lower extremity The serum albumin level was raised temporarily on four separate

occasions with partial loss of œ le…
addition the pat…
injections
The operatio…
persistent œde…a and depression … to a normal life, despite … value
level A marked increase in body weight also occurred which persisted

The fact that this patient developed hæmorrhages from
proven varices and that her portal pressure was found to be
over 300 mm of water indicates that elevated portal pressure
was probably present throughout the pre operation period
However, it was not enough in itself to cause ascites as long
as the serum albumin level was normal When the albumin
fell following hæmorrhage, ascites developed with very little
œdema Raising the albumin level by albumin injections, or
more slowly by oral nutritional therapy, caused the ascites
to disappear After spleno renal anastomosis with elimination
of the increased portal pressure, the hypoalbuminæmia
…h developed did not alone produce ascites, although
…na was prominent In this patient the combination of
…ased portal pressure and hypoalbuminæmia was neces
sary for ascites to form Her cirrhosis was of the post
necrotic type as proven by biopsy at two laparotomies
Antidiuretic material could not be found in her urine when
tested in the hydrated rat one year prior to the spleno renal
anastomosis Her entire picture appeared more simple than
that usually encountered in patients with the alcoholic type
of cirrhosis and severe ascites Hypoalbuminæmia seemed
to be more directly related to the ascites, at least the relation
ship could more readily be demonstrated

Exactly how hypoalbuminæmia exerts an effect in the
retention of fluid both in this type of patient with cirrhosis
and in patients with nephrosis is not clear It is certainly
a more complex story than simply that the colloid osmotic
pressure in the blood is reduced and therefore fluid escapes
into the tissues The work with intravenous albumin has
demonstrated that albumin moves freely from the serum

into the tissue fluids and that an equilibrium is rapidly established To raise the serum albumin level by 1 gram per cent means raising the albumin level in ascitic fluid or œdema fluid by a similar amount The concept of a rigid vascular wall impermeable to colloids such as albumin is not tenable

Recently, we have been interested in the effects of albumin administered intraperitoneally to patients with cirrhosis and ascites, such as the one described above Such albumin is rapidly absorbed into the blood stream, and it is possible to raise the serum albumin level markedly by this means without increasing the volume of ascitic fluid In two patients a loss of ascitic fluid was actually obtained by administering albumin intraperitoneally Osmotic pressure measurements indicated that when equilibrium was established in approximately twenty four hours following the injection, the osmotic pressure of the serum had risen to the same degree as the osmotic pressure of the ascitic fluid A diuresis was produced because of an increase in plasma volume that developed secondarily to the rise in the serum albumin level These effects offer additional evidence for the rapid interchange of protein between serum and tissue fluids

The critical factor, however, in the overall retention of fluid is the retention of sodium by the kidney The relation between hypoalbuminæmia and sodium retention is a dominant problem under investigation in patients with nephrosis as well as those with cirrhosis Certainly the reduced effective blood volume is one of the factors in the retention of sodium, but there appear to be many others The observations of Farnsworth and Davidson and co workers on the extreme sodium retention by the kidney in many patients with the alcoholic type of cirrhosis are particularly interesting Eisenmenger has recently demonstrated that over wide ranges of sodium intake the sodium excretion by the kidney in such patients remains extremely low, and as a result, the amount of ascitic fluid laid down is directly proportional to the sodium intake The extreme sodium retention appears to result from the stimulus of a constant

state of dehydration and correlates with a frequently reduc serum sodium level, despite a high intake of sodium The effects are undoubtedly mediated through the adrenal a ndeed, Luetscher has recently separated increased amoun f salt retaining steroids in the urine of certain patients wit evere sodium retention The force to retain sodium i sentially protective in nature, but it reaches extrem oportions in the alcoholic type of patient with cirrhosis, coming more pronounced the longer the ascites persists In such cases, it is often very difficult to counteract, even when such forces as hypoalbuminæmia and increased portal pressure, which originally initiated the sodium retention, are reversed This represents one of the reasons for the relatively poor therapeutic effects of intravenous albumin in such patients, in contrast to the group mentioned earlier, where the sodium retention never reaches such extreme proportions and where the relation between hypoalbuminæmia and portal pressure is more apparent

THE PATHOGENESIS OF ASCITES

POUL IVERSEN

THE current view of the pathogenesis of ascites is as follows If ascites is to develop the hydrostatic pressure in the blood capillaries in the abdominal region must be higher than the difference between colloid osmotic pressure in plasma and ascitic fluid + the hydrostatic pressure in the abdominal cavity The factors that enter into this relationship will again be influenced by the permeability of the capillaries by the electrolytes, by blood volume changes and by the antidiuretic hormone

The most important factors in the formula are no doubt the hydrostatic pressure in the blood capillaries and the colloid osmotic pressure of the blood The hydrostatic pressure in the abdominal cavity is less important Its maximum value is 100 mm of water regardless of the degree of ascites As it indirectly increases the hydrostatic pressure through pressure upon the veins in the abdominal cavity it cannot be a limiting factor for the development of ascites

The main points in this view of the pathogenesis of ascites have been formulated by Poul Iversen in 1928, based on a number of direct measurements of colloid osmotic pressure in blood and ascitic fluid

In the third medical department, Kommunehospitalet, Copenhagen, we have in recent years studied the pathogenesis of ascites in subchronic atrophy of the liver In these investigations we have used direct measurements of colloid osmotic pressure of serum, serum protein analyses and pathological anatomical studies Subchronic atrophy of the liver is characterized by a marked reduction in liver parenchyma and a pronounced failure of most liver functions The relation of this disease to typical portal cirrhosis is not clear As far

as the duration of symptoms is concerned we have not been able to demonstrate any difference between groups of these two liver diseases observed in Kommunehospitalet in recent years.

Direct Measurements of Colloid Osmotic Pressure of Serum, and Serum Protein Analyses

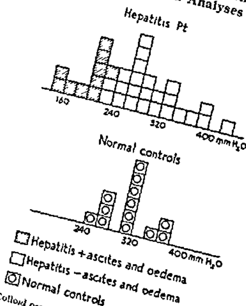


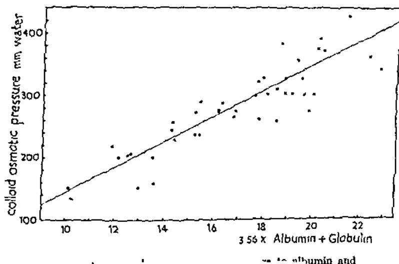
Fig 1 Colloid osmotic pressure in patients with hepatitis and in normal controls
(M Bjorneboe C Brun and F Raaschou Arch int Med 83 539-546 1949)

Fig 1 shows the distribution of measurements of colloid osmotic pressure in a number of cases of hepatitis mostly cases of a severe type which at autopsy proved to be sub chronic atrophy of the liver It is evident from this figure

that there is a sharp limit between cases with and without ascites The limit is a colloid osmotic pressure of 220–240 mm water.

Fractional analyses of serum proteins (using ammonium sulphate precipitation) Fig. 2 shows the relation between colloid osmotic pressure of serum and serum *protein fractions* according to the formula —

Colloid osmotic pressure $= k \times$ (*3.56 albumin* + *globulin*) (in mm. water)



... albumin and

Med, 83,

where k is 16 This formula was found to fit our data best The standard deviation is 33.

To find the relation between the occurrence of ascites and the concentrations of serum albumin and globulin we have in Fig 3 plotted albumin against globulin for a large number of cases of hepatitis, with and without ascites. Each circle represents one case It is evident from the figure that there

is a sharp limit between cases with and without ascites and that this limit can be expressed by the formula of the straight line —

3 5 albumin +globulin = 14 2

that is when in a given case of hepatitis the sum 3 5 albumin +globulin is below 14 2 the patient has ascites

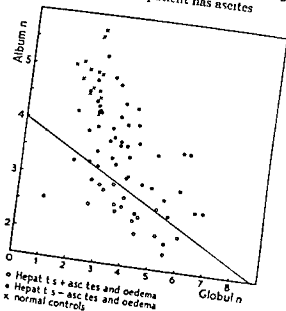


FIG 3 Relation between values for serum albumin and those for serum globulin

(M Bjørneboe C Brun and F Raaschou. *Arch int Med* 83 539–546 1949)

These investigations have shown that we can find a sharp limit between cases of hepatitis with and without ascites when we consider the colloid osmotic pressure measured directly in serum and also when we consider the concentrations of the serum protein fractions We conclude from these

results that in this type of liver disease (subchronic atrophy of the liver) the strength of the colloid osmotic pressure is the most important factor in the pathogenesis of ascites

From Fig 3 it can be seen that the concentration of albumin

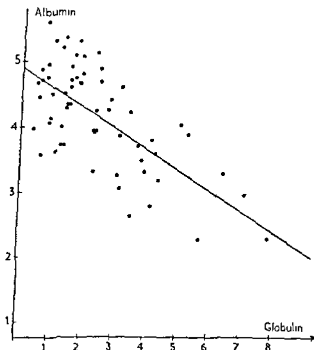


FIG 4 Relation between albumin and globulin in sera from rabbits immunized with polyvalent antigen The line through the points has the formula 3 2 Alb + Glob = 15 6
(*Acta path et microbiol Scand*, 22, 323–334, 1945)

decreases when the concentration of globulin increases There is a considerable dispersion of the points but the main tendency is obvious We have considered the possibility that this might be an indication of a regulatory mechanism trying to keep the colloid osmotic pressure constant while the concentrations of albumin and globulin fluctuate Ascites might

then be the result of an insufficiency in this regulatory mechanism. We have gone into this question by studying the relationship of albumin and globulin in rabbits where the concentration of serum globulin has been increased actively

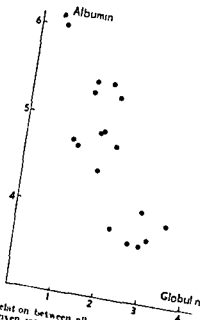


Fig. 5. Relation between albumin and globulin in sera from rabbits given intravenous injections of globulin solution. (Acta path. et microb. Scand. 22, 3, 331, 1945.)

(by hyperimmunization) or passively (by injection of large amounts of rabbit serum globulin).

Figs. 4 and 5 show the results of these experiments.

The protein analyses have been carried out at junctures where there was no difference in blood volume between states with low and high globulin level. Ammonium sulphate precipitation has been used for determining the serum protein

fractions The figures demonstrate the same inverse relationship between albumin and globulin as the hepatitis material We therefore believe that this regulation mechanism exists but as the dispersion of the points in these graphs is rather large we think that this mechanism is not the only one for determining the relation between albumin and globulin in serum

Pathological Anatomical Investigations

We have concluded from our investigations that the level of the colloid osmotic pressure in serum is of decisive importance for the occurrence of ascites in our patients This result may be due to the fact that our material consists of cases of subchronic atrophy of the liver and not of cases of portal cirrhosis We have studied this question by examining 108 autopsied cases of subchronic atrophy of the liver and 76 autopsied cases of portal cirrhosis all autopsied during the same period In the first series of cases 74 per cent had ascites in the other series 54 per cent We have studied these cases of ascites more closely Among the cases with subchronic atrophy and ascites 50 per cent had œsophageal varices whereas 73 per cent of the cases of portal cirrhosis with ascites had varices Besides the degree of development of the varices and the *incidence of rupture of the varices were* higher in the group with portal cirrhosis We conclude from these investigations that the portal hypertension measured by the frequency and degree of development of œsophageal varices is higher in portal cirrhosis than in subchronic atrophy of the liver It ... ore lo... that the strength of the c... pre... important factor for the dev... f ... atrophy of the liver than ... ent with the observation ... se ascites

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in the abdominal cavity, the electrolytes, changes in blood volume and the antidiuretic hormone We have not given any personal contributions to these problems It seems to be proved that there is an increased permeability of the capillaries in cirrhosis of the liver with ascites Patek and co-workers have demonstrated that injected serum albumin quickly passes from the blood to the ascitic fluid Hiller and co-workers have shown that one may find rather often an increased blood volume in cirrhosis, presumably due to enlargement of the liver and the spleen and the highly developed collateral circulation Several authors have found high values for antidiuretic hormone in the urine of patients with cirrhosis and ascites The importance of the electrolytes is best demonstrated by the therapeutic effect of salt-free diet in cases of cirrhosis with ascites

GENERAL DISCUSSION

CH WUNDERLY I would like to say a few words about the formula iven by Dr Iversen for the measurement of colloid osmotic pressure —

$$COP = K(3\ 5\ A + G)$$

here K is a constant, A is albumin and G globulin This woul[d] nstitute a linear relation between the concentration of the protein d the colloidal osmotic pressure Now the latter is also dependent hydration of the protein molecule and therefore on its surface and ...eral structure (loose or tight) For these reasons osmotic pressure increases faster than the concentration so that the function is not linear At low protein concentration the colloid osmotic pressure varies quickly, but in high concentration the colloid osmotic pressure varies regions of hydration of adjacent molecules because of the mutual effects of the reaches an upper limit As measurement of colloid osmotic pressure can only be studied on highly purified proteins it is difficult to know how albumin in cases of hepatitis compares with normal albumin Pure normal albumin gives 3 5 times the pressure of globulin and is there fore more important roughly by a factor of 3 5 If in hepatitis albumin has a smaller molecule than normally then its surface would be smaller and there would be more molecules per volume weight This albumin would exert a higher colloid osmotic pressure than normal albumin and would certainly have a tendency to leave the body We know from Bence-Jones uroprotein (34 000) and experiments with hæmoglobin (69 000) that if in hepatitis the molecular weight of albumin should sink below 68 000 it would leave the body Therefore it is unlikely that albumin in hepatitis has a smaller molecular weight but rather has an

increased molecular weight, perhaps 80 000 The surface of such a

more complicated one The postulated regulatory

relationship between serum albumin and serum globulin cannot be a very sensitive one since the dispersion of the points in our graphs is so large

OBSERVATIONS ON THE SURGICAL PROBLEMS IN PORTAL HYPERTENSION*

A I S MACPHERSON

SINCE it is impossible to cover all the surgical problems in portal hypertension in the time at my disposal I propose, with your permission to consider three aspects only—the lesion encountered in 128 cases of portal bed obstruction and its ætiology, the principles of treatment of portal hypertension, and a review of the place of porta systemic venous shunts in the treatment of ascites For the opportunity to work with them and for permission to make use of their case material I am deeply indebted to Dr Arthur H Blakemore and to Sir James Learmonth

In the fœtus there are valves in the tributaries of the portal vein which may persist in the gastric veins during the early years of life and be sufficiently competent to prevent reflux of blood In the adult however the valves have degenerated [though Learmonth (1950) doubts this] and are no longer competent The effects of any obstruction in the portal system will, therefore be distributed over the whole area distal to the obstruction and will vary with the rate of its onset, the degree of obstruction and the site of the block

Complete obstruction of sudden onset whether it be wide spread or be critically situated to block the main drainage from part of the alimentary canal, is followed by intestinal infarction and by an acute illness which is often rapidly fatal Such a lesion rarely gives rise to chronic portal hypertension Slow obstruction either complete or partial leads to (1) congestion in the area beyond the obstruction The evidence for this in the chronic case is found in the enlargement of the

*Some of the work reported in this paper was done during the tenure of a Rockefeller Foundation Travelling Fellowship

Extra-hepatic Block

In the combined series of 128 cases there are 30 of extra hepatic portal bed block (Table I) A O Whipple's (1945) famous case of cicatricial occlusion of the splenic vein gave rise to the idea that obstruction in the splenic vein alone was common, but in this series it has been possible to demonstrate it with certainty in only two cases In nine cases the obstruction was in the portal vein, in eight it was probably there, and in six cases a partial or complete portal vein thrombosis was associated with cirrhosis of the liver and was probably secondary to it In 11 cases the site of obstruction was not determined The thrombus in the portal vein had become organized and canalized in two instances, but it was evident that no significant volume of portal blood was reaching the liver through these channels In one case the portal vein was blocked with carcinoma Congenital abnormalities were found in seven cases A complete atresia of the portal vein was present on four occasions In two cases there was congenital dilatation of the splenic vein and the proximal end of the left gastric vein, and in one case the veins were small and multiple A cavernous angioma a true vascular tumour of the portal vein at the hilum of the liver, was found once

Table I

EXTRA HEPATIC OBSTRUCTION

A *Site of Obstruction*			
Number of cases *30*			
Portal vein	—*proved*		9
	probable		8
Splenic vein	—proved		2
Undetermined			11
Cirrhosis+portal vein thrombosis			6
B *Type of Lesion*			
Organized thrombosis	—alone		2
	—with cirrhosis		6
Congenital abnormality	—atresia		4
	varicosity		2
	small multiple veins		1
Cavernous angioma of portal vein			1
Obstruction by carcinoma			1

It is difficult, even at operation to determine the site of an extra hepatic block Its existence may be suspected by the normal results of liver function tests before operation and by the appearance of the liver at the time of operation. Pressures taken in a radicle of the splenic vein and in a radicle of the superior mesenteric vein under comparable conditions will confirm the presence of portal obstruction and may help to localize it Portal phlebography has proved disappointing, but it may be possible to get better results by passing a catheter along the splenic vein and injecting the opaque medium through it when it is arrested Exploration of the sub hepatic area may reveal the presence of a collateral circulation of the so called cavernomatous type a finding which strongly suggests the presence of portal vein block

Principles of Surgical Treatment

I would like to be deliberately provocative at this point, and to define the place of surgery in portal hypertension as the treatment of bleeding and not of any other sign or symptom In fact I would go so far as to say that the presence of severe hepatic dysfunction is an indication to delay operation if at all possible The aim of surgical treatment is twofold first to attempt to reduce the portal blood pressure secondly and possibly more important to divert the blood from the dangerous cardio-œsophageal area The choice of operation must depend upon the pathology the site of the obstruction and the age and general condition of the patient Splenectomy alone should relieve most cases of splenic vein thrombosis Where the obstruction is in the portal vein it is advisable to divert the blood by anastomosis of the splenic to the renal vein or of the proximal cut end of the superior mesenteric vein to the inferior vena cava Unfortunately there are many difficulties These cases are often young and their veins are small and usually abnormal, double thin or abnormally thick or showing patches of sclerosis Frequently therefore splenectomy alone is done as an operation of necessity and not from choice In three

cases total gastrectomy was done by Dr David Habif Over a period of six to eighteen months bleeding did not occur, but the nutritional difficulties which followed the operation were so great that I would advise it only as a last resort, and certainly not in a cirrhotic In the cirrhotic the operation of choice is the porta caval anastomosis The biochemical effects of diverting the portal blood from the liver are very much less than animal experiments lead one to expect, probably because the larger proportion of the portal venous blood is already not coming in contact with the liver cells and the organ has therefore had a chance to adapt itself to *the circulatory changes which operation completes*

Porta-systemic Anastomosis in the Treatment of Ascites

Porta systemic venous anastomosis was performed in 13 cases of cirrhosis of the liver in which ascites was the main presenting feature, and in one case in which it appeared for the first time after operation The existence of portal hyper tension was proved in 12 cases—in nine by direct measurement of the portal blood pressure at operation, and in four by the presence of an enlarged spleen, and of œsophageal varices or other large venous collaterals In two cases (Nos 6 and 8), the spleen was enlarged moderately, but there were no *œsophageal varices and the portal pressure was not recorded*

Absolute evidence of the fate of the anastomosis was obtained at necropsy in nine cases On four occasions the shunt was found blocked by recent thrombus formation In five cases the shunt was open, but in Case 10 it was only about one quarter the calibre of the two adjacent veins Shrinkage of the spleen, or of œsophageal varices after porta caval anastomosis, provided circumstantial evidence of a patent shunt in Cases 11 and 14, and in three patients (Nos 8, 12 and 13), the progress of the disease suggested that the anastomosis was open and *functioning The period of* follow up in the surviving patients has been at least twelve months

Results

In these 14 cases porta systemic venous anastomosis was followed by —

Death in the post operative period	7 cases
No effect on the progress of the disease	4 cases
Improvement after operation	3 cases

A patent anastomosis was found at necropsy in four of the seven post operative deaths. In six cases severe liver dysfunction was demonstrated by estimation of the plasma proteins, serum bilirubin, alkaline phosphatase, and the cephalin cholesterol flocculation (Table II). Five patients died within a week of operation and in all of them liver failure was the direct cause of death. In Case 3 and Case 5 it was precipitated by hæmorrhage from œsophageal varices and in Case 4 by the effects of a long and difficult operation. In Case 7 widespread splenic, portal and superior mesenteric venous thrombosis was found at necropsy. Ascites was again evident in Case 6 before his death on the tenth post-operative day. At post mortem examination there were no œsophageal varices: the cause of death was bleeding from an eroded vessel in the base of an acute gastric ulcer.

The condition of the patient has not been improved or the progress of the disease strikingly affected, by porta systemic venous anastomosis in four cases (Table III). After operation, ascites developed for the first time in one patient (Case 10), and recurred in three. Two patients were dead within a year of operation. The cause of death in Case 10 was progressive liver failure. A large subhepatic abscess and a closed shunt were found at post mortem examination of Case 9 63 days after a porta caval anastomosis. The two survivors were —

Case 8, a man aged 49. Five months before admission to hospital he developed ascites which did not respond to treatment by paracentesis, mercurial diuretics and the prohibition of alcohol. A vitallium tube was used to make a

end to side porta caval anastomosis on 24th April 1945 (Dr A H Blakemore) The liver was small and its lobular pattern distorted Ascites recurred and required paracentesis at about ten day intervals for four months after operation During the succeeding two years he gained 50 lb in weight He was free of ascites and œdema for nearly four years In June, 1949, his doctor reported that ascites had again developed

Case 11, a man aged 42, complained of a 25 lb weight loss over a five year period Between December 1947, and September, 1948, there were three episodes of melæna On admission he was jaundiced and lean, with a swollen abdomen and great enlargement of the liver and spleen On 15th October, 1948 an end to side porta caval anastomosis was performed by Dr A H Blakemore Liver biopsy showed active chronic hepatitis Ascites and pleural effusion recurred for three months after operation, despite high protein diet and intravenous salt poor albumen Thereafter he remained free of fluid for eight months The spleen decreased markedly in size, but liver enlargement and jaundice persisted In November 1949, cortisone, given in an attempt to diminish the interstitial inflammation in the liver induced temporary regression of the jaundice decrease in the size of the liver, and a massive ascites which disappeared within five days of stopping the drug (Hanger, 1950)

In three cases a noticeable decrease in the rate of ascitic fluid formation has followed porta systemic venous anastomosis

Case 12 a girl aged 18 had a prolonged attack of infectious hepatitis in 1941 The first hæmatemesis in 1945 was followed by a transient ascites the second in 1946 by coma for two days and ascites which responded to intravenous albumen During the succeeding eight months melæna occurred on three occasions and ascites was constantly present, though not to a degree requiring paracentesis The liver function tests are recorded in Table IV At operation (13th May, 1947, Dr A H Blakemore) a small nodular liver was found and

Table II

DEATHS IN THE POST-OPERATIVE PERIOD WITHIN ONE MONTH OF OPERATION

Case No.	Age	Symptoms			Liver Function Tests					Total Blood [illegible]	Day of death after opn.	State of anastomosis
		Ascites	Bleeding	Duration (months)	Albumen g/100 cc	Globulin g/100 cc	Bilirubin mg/100 cc	Alkaline phosphatase	Cephalin flocculation			
1	39			3	1.9	4.4	4.6	6.6	+(3–4)	30	3	Closed
2	58			10	2.4	2.6	0.7	6.2	+(2)	39	5	Open
3	10	+		3	2.6	3.73	—	—	+(4)	—	6	Closed
4	48	+	+	5	3.07	2.56	<1.0	13.0*	—ve	—	2	Open
5	44			16	2.80	2.55	—	11.0*	+(4)	—	5	Open
6	47			13	3.0	1.8	<1.0	11.5	—ve	—	10	Open
7	40		+	30	2.8	2.0	Trace	3.8	+(3)	38	10	Closed

*These results are expressed in King Armstrong units. All others are in Bodansky units.

Table III
RFCURRENCE OF ASCITES AFTFR OPFRATION

Case No	Age	Symptoms			Liver Function Tests					Portal B P (cm saline)	Remarks
		Ascites	Bleeding	Duration (months)	Albumen g 100 cc	Globulin g / 100 cc	Bilirubin mg / 100 cc	Alkaline phospha tase	Cephalin floccula tion		
8	49	+		5	2 9	3 0	0 8	10 2	—ve		See text
9	40	+	+	18	3 0	2 8	<1 0	4 4	—ve	28 5	Died 63 days post op Shunt closed
10	38			7	3 5	4 0	1 5	6 0	+(3)	40 0	Died 10 mth post op
11	42	+	+	7	3 4	4 1	2 1	19 4	+(3)	23 0	See text

Table IV
IMPROVEMFNT AFTER OPERATION

Case No	Age	Symptoms			Liver Function Tests					Portal B P (cm saline)
		Ascites	Bleed ng	Durat on (months)	Albumen g / 100 cc	Clobulin g / 100 cc	B lirubin mg / 100 cc	Alkaline phospha tase	Cephalin floccula tion	
12	18	+	+	17	3 6	2 7	1 3	6 1	+(3)	39
13	38	+		33	3 7	2 2	<1 0	16 2	+(3)	25
14	◡0	+		12	3 1	2 6	0 7◡	13 0	+(3)	28

splenectomy and end to side spleno-renal anastomosis were done There has been no recurrence of bleeding or of ascites, notwithstanding a fall in the plasma proteins five months after operation to albumen, 2 6, and globulin 2 0 g per cent Œdema appeared in the legs and has persisted down to pre

Case 13 a woman aged 38 Subacute hepatic necrosis in December, 1944 was followed by ascites which required frequent paracentesis and was only temporarily relieved by sapheno peritoneal anastomosis in February 1945 and button drainage of the peritoneal cavity in September, 1946 The liver was then reported to be large and soggy In September 1947, the ascites was made worse by 150 g of albumen intravenously The operation of splenectomy and end to side spleno-renal anastomosis was performed on 10th October, 1947 (Dr A H Blakemore) The liver was small and nodular and the portal pressure 20 cm of saline Ascites has not recurred in the two years since operation

Case 14 a man aged 51 who admitted to a considerable daily consumption of spirits complained of loss of appetite and weight for eight months before leg œdema and ascites appeared in July 1948 A 2 500 calories high protein high vitamin diet with methionine 3 g per day and no alcohol improved his general health but, even when supplemented by mercurial diuretics and intravenous albumen did not influence the formation of ascitic and œdema fluid On 24th April 1949 an end to-side porta-caval anastomosis was performed (Dr A H Blakemore) The portal pressure was 23 cm of saline The liver was small and showed severe lobular distortion On ordinary ward diet plus protein supplements and an unrestricted salt intake ascites and œdema recurred Mercurial diuretics again were ineffective but a diet containing 180 g of protein and 1 5 g of sodium chloride caused a loss of 6 kg in weight over a three week period This régime has been continued at home There has been no detectable ascites, and no œsophageal varices can

be seen on barium swallow, but œdema of the legs has persisted

Assessment of Results

The prognosis of ascites in cirrhosis of the liver varies with the circumstances of its development In a person who shows no other signs of liver disease, it may appear after an episode of bleeding, an operation or an acute general infection, each of which produces a temporary condition of hepatic insufficiency If the impact of such episodes on an already damaged liver is severe, coma and death may ensue, but in most cases the ascites is transient and its disappearance may be hastened by infusion of concentrated albumen (Kunkel *et al*, 1948) The occurrence of ascites in the cirrhotic patient who already presents evidence of profound liver damage—loss of weight, anorexia, jaundice, mental apathy, or grossly abnormal liver function tests—is of serious prognostic significance Cates (1943) reported that of 53 patients who had survived one month after the onset of ascites 62 per cent were dead within a year In a series of 162 cases of cirrhosis analysed by Henrikson (1936) the duration of life after ascites appeared averaged 12 8 months In six of the seven patients who died in the immediate post operative period (see Table II, page 153) and in all those who were not benefited by operation there was evidence of severe liver dysfunction In one patient (Case 5 Fig 1) on a high protein diet (140 g daily) without restriction of salt the liver was unable to form sufficient albumen to balance the loss into the ascitic fluid (about 1 g per day) and maintain its level in the plasma In such circumstances quite minor surgery may precipitate an hepatic crisis Morison (1896 1912), Talma (1898) and Turner (1906) emphasized that toxæmia, jaundice and failure to withstand at least two paracenteses were contraindications to operation in cirrhosis

After porta systemic anastomosis in five cases, the fall in portal pressure averaged 10 cm of saline (range 5-16 cm) If the presence of portal hypertension were the most important

contributory factor in the development of ascites, such a reduction in portal pressure should be followed by an unequivocal diminution in the rate of ascitic fluid formation This did not occur in three cases In Case 8, the state of the anastomosis is not known, but in Case 11 there is circum

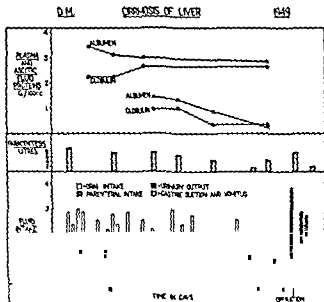


Fig 1 Records of fluid balance and of plasma and ascitic fluid proteins of patient with cirrhosis on a high protein diet.

stantial evidence of a patent shunt in the progressive diminution in the size of the spleen Fluid continued to form, however, for three months after operation and nine months later the mild upset of salt and water metabolism caused by the administration of cortisone induced an ascites which disappeared five days after the drug was withdrawn In Case 10 ascites appeared for the first time after a shunt which

hepatic degeneration, venous anastomosis may be followed by relief of ascites

On two occasions, however, ascites disappeared after a successful porta systemic shunt, but peripheral œdema persisted and seemed to become more resistant to treatment These observations support the view that ascites in the patient with cirrhosis of the liver is part of a generalized fluid retention *The raised portal pressure determines the* localization in the peritoneal cavity of the retained fluid, but contributes little to its actual formation

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GENERAL DISCUSSION

that when we are dealing with a large

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of hydrostatic factors

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His explanation would

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pericarditis where you have obstruction to the entry of blood into the heart He has produced obstruction to the inferior vena cava above the liver, and obtained the effects noted by Dr Charles Bolton many years ago He then suggests that dilated liver lymphatics transfer fluid somehow into the peritoneal cavity The dilated lymphatics should I imagine communicate with the thoracic duct and the fluid should keep in circulation that way I don t see that that accounts for

[illegible]

retention in patients who already had ascites but I would like to hear from him more about the controls in people with hepatic disease without ascites

[illegible]

these as ... has shown are actual anatomical abnormalities at the porta hepatis but other things must go on inside the liver distorting the vascular arrangement long before actual scar contraction

[illegible]

congestion of the small intestinal mucosa in the absence of actual varicosities which accounts for some of these bleedings We also know that with a liability to epistaxis and sometimes to actual purpura, the patient with severe liver disease may also have some hæmorrhagic diathesis

Clinically the patient who has very severe hepatic failure will often

[illegible]

us up with the recent work of Shorr

I am simply emphasizing that the problem is extremely complex

DISCUSSION STUDIES ON "ALCOHOLIC" CIRRHOSIS AND CHRONIC RECURRENT FLUID RETENTION AND ASCITES

T C CHALMERS

[illegible] hat Dr Kunkel has said by [illegible] Drs George Gabuzda, H S [illegible] (1950) in the Thorndike *Memorial Laboratory on patients* with "alcoholic" cirrhosis and chronic recurrent fluid retention and ascites I would like to summarize first by saying that it has been said quite frequently that the defect is either in poor renal function, resulting in the retention of salt, or else that it is in poor metabolism of hormones by the liver, resulting in retention of salt by the kidney However, it may be that the kidney is working beautifully, and the homeostatic mechanism of the hormones is working beautifully, and that if they did not, the patient would die of an Addisonian like crisis I think the best way to show that problem is to consider the importance of the paracentesis in continuing what is referred to as the abnormal sodium retention of cirrhosis Thus it can be shown that the urinary sodium retention is necessary to compensate for the massive amounts of sodium lost by way of the peritoneal cavity

Fig 1 shows the balance study of a patient with chronic ascites who had 13 litres of fluid removed His serum sodium was around 138 at that time, and that would mean that about 120 g sodium chloride was removed within a few hours from his peritoneal cavity After paracentesis there is a rapid gain in weight over the first three days accompanied by a definite drop in urine output, then a levelling off with a slower rate of weight gain There is a rise in the hæmatocrit, showing that he is becoming hæmo concentrated as the fluid

ours into his ascitic cavity, and there is a drop in the serum odium, suggesting that he is retaining water in excess of alt He doesn't put out any sodium in his urine because uch a tremendous amount is being lost into his ascitic fluid ssociated with this external loss of sodium is a low serum

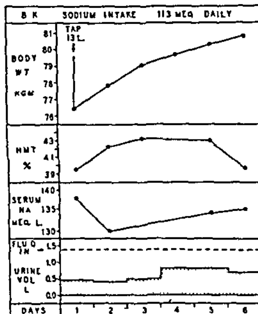


Fig 1 Effect of paracentesis on the hæmatocrit serum sodium and fluid balance

odium to which the patient with chronic ascites becomes ccustomed

Fig 2 is shown to emphasize that patients with massive ascites have some œdema which we believe is related to pressure of the ascitic fluid on the inferior vena cava When apped the patient doesn t gain much weight but his ascites

DISCUSSION STUDIES ON "ALCOHOLIC" CIRRHOSIS AND CHRONIC RECURRENT FLUID RETENTION AND ASCITES

T C CHALMERS

I would like to supplement what Dr Kunkel has said by reporting some studies done by Drs George Gabuzda, H S Traeger and Charles Davidson (1950) in the Thorndike Memorial Laboratory on patients with "alcoholic" cirrhosis and chronic recurrent fluid retention and ascites I would like to summarize first by saying that it has been said quite frequently that the defect is either in poor renal function, resulting in the retention of salt, or else that it is in poor metabolism of hormones by the liver, resulting in retention of salt by the kidney However, it may be that the kidney is working beautifully, and the homeostatic mechanism of the hormones is working beautifully, and that if they did not, the patient would die of an Addisonian like crisis I think the best way to show that problem is to consider the importance of the paracentesis in continuing what is referred to as the abnormal sodium retention of cirrhosis Thus it can be shown that the urinary sodium retention is necessary to compensate for the massive amounts of sodium lost by way of the peritoneal cavity

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pours into his ascitic cavity, and there is a drop in the serum sodium, suggesting that he is retaining water in excess of salt He doesn t put out any sodium in his urine because such a tremendous amount is being lost into his ascitic fluid Associated with this external loss of sodium is a low serum

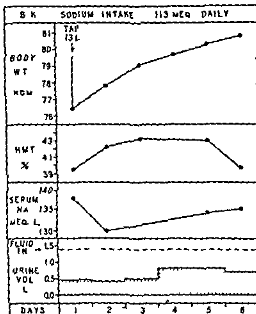


FIG 1 *Effect of paracentesis on the hæmatocrit, serum sodium and fluid balance*

sodium to which the patient with chronic ascites becomes accustomed

Fig 2 is shown to emphasize that patients with massive ascites have some œdema which we believe is related to pressure of the ascitic fluid on the inferior vena cava When tapped the patient doesn't gain much weight but his ascites

recurs just as much because the œdema fluid is mobiliz and, because of the increased portal pressure, is deposit in the abdominal cavity There is an estimated decrease plasma volume of about 14 per cent The hæmoglobi

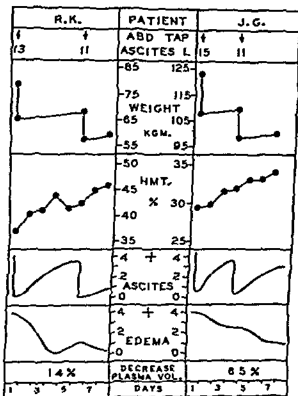


FIG 2 Effect of paracentesis on the body weight hæmatocrit and estimated ascites œdema and plasma volume in a patient on a normal salt intake.

parallel the hæmatocrits, which suggests that it is not just a change in size of the red cells but is an actual hæmo concentra tion So the patients receiving frequent paracenteses have decreased plasma volumes, are hæmo concentrated, and have a lowered serum sodium

Fig 3 is a two month's balance study of one of the same patients with chronic ascites At the beginning he has a lot of ascites, some œdema, a low serum albumin, serum sodium around 134—he has had taps in the past—and his hæmatocrit

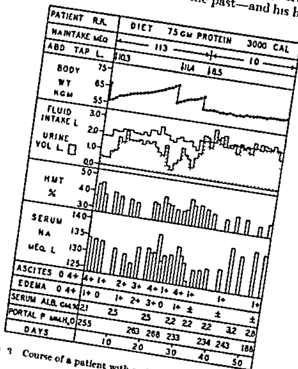


FIG 3 Course of a patient with ascites on high and low sodium intakes.

is around 38 He is tapped three times His urinary volume falls his hæmatocrit rises, serum sodium falls, and then as ascites accumulation slows down, his urinary volume comes up, his hæmatocrit drops down nearer to his base line level Dr Gabuzda's data suggest that these patients have a definite

anæmia and that their hæmatocrits are kept falsely elevated by the hæmo-concentrating effects of the repeated paracentesis Thus they may not have an increased blood volume as has been shown by the Evans blue method, but their blood volumes may in fact be restricted For the first period the patient in Fig 3 was on a diet containing about 7 grams of *sodium chloride, 113 milliequivalents* After he has had several taps and his œdema has disappeared he is put on a diet containing 10 milliequivalents of sodium, about 0 6 grams of sodium chloride, per day His *weight stays the same,* his urinary volume comes up, his hæmatocrit stabilizes his serum sodium gradually rises If this chart were continued through the next six months it would show a gradual gain in weight, caused by an accumulation of flesh, not fluid, accompanying a gradual increase in urine output, a gradual stabilization of the hæmatocrit at about 34 or 35 During this time *his serum sodium gradually rises until it reaches normal* because he is no longer receiving paracenteses His portal venous pressure was measured by putting a needle in a collateral abdominal vein, obstructing the vein, cephalad, and assuming that this was somewhere near the true portal pressure It seemed to decrease as he got better Two or three months later his urinary sodium, previously very low, began to rise *slowly and now, about eight months later, the* patient is beginning to eat salt and to put it out in his urine Apparently the patients maintained successfully on a low sodium diet eventually are able to eat sodium without ascites reaccumulation The explanation is not apparent

Mr Macpherson has said that he wants to be most dogmatic in saying that surgery has no place in the treatment of ascites I would like to be equally dogmatic and say that we believe that no type of surgery, even paracenteses, has any place in the treatment of ascites once the patient has been stabilized *by the first one or two paracenteses necessary* to remove the fluid present on admission, including the œdema fluid But the diet has to be very low in sodium, less than 0 6 g sodium chloride, and it has to be maintained, in some instances for

prolonged periods The patients don't seem to mind because they appreciate so greatly not being tapped any more If specially prepared salt free products are used, such a diet may contain 75–100 g protein

Both Dr Iversen and Dr Kunkel have pointed out the apparent difference between the patho physiology of the ascites in the patient with post hepatitis cirrhosis and the patient with chronic Laennec's cirrhosis I would like to suggest that this difference may be merely a reflection of the duration of the disease

The major differences alluded to are (1) the disappearance of the ascites in post hepatitis cirrhosis on the administration of concentrated salt poor human albumin, and the lack of response in chronic Laennec's cirrhosis, (2) the presence of sodium in the urine and a normal concentration in the blood in the post hepatitis patients, in contrast to the low serum sodium and marked sodium retention in the patient with chronic ascites accompanying Laennec's cirrhosis The first difference may be explained by the retention of administered albumin in the vascular compartment in one type and its filtration through an injured capillary membrane in the other And the second difference may be explained by the chronic sodium depletion of the patient with Laennec's cirrhosis who has had many paracenteses The patient with post hepatitis cirrhosis may well die of hæmorrhage or coma before there has been much change in the permeability of the capillaries in his portal circulation or much time for the constant reaccumulation of ascites and loss, by paracentesis, of sodium

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DISCUSSION FACTORS IN THE PRODUCTION OF ASCITES

ROBERT M KARK

We believe that there are many factors which, when the act together, produce ascites in individual patients witl cirrhosis of the liver Moreover, if a combination of differen factors are producing ascites in a patient, removal of one o the factors may clear up the ascites

Our studies show that *in many alcoholic patients with* cirrhosis an adrenal dissociation exists, with very low 17 *ketosteroid output and high* cortin *levels in the urine (Table I) This hormonal situation is reflected in their body con* figuration Recently, we injected cortisone into patients with a low sodium intake, and when we withdraw cortisone, we *observed a sodium diuresis*

Table I

24-Hour 17 Ketosteroids and Cortin Excretion in the Urine from Six Patients with Cirrhosis of Liver

No	Patient	Age	Sex	Blood Pressure	Urinary Cortin mg per 24 hours	Urinary 17 Ketosteroids mg per 24 hours	Eosinophiles per MM3
1	L F	58	M	116/68	4 5	1 6	100
2	*T W*	*58*	*M*	*118/62*	*3 8*	*0 75*	*140*
3	K K	54	M	112/72	3 3	1 6	343
4	A M	*32*	*M*	*98/56*	*4 3*	*1 2*	*66*
5	P W	52	M	108/62	5 7	2 2	121
6	B F	60	M	128/70	3 8	1 4	143

Fig 1 shows the effect of a low sodium diet in preventing the re accumulation of ascitic fluid Note that his "dry body weight after the paracenteses was about 55 kg He is a big man that is big boned, and his best body weight when

he was well was about 85 kg He has therefore lost a considerable amount of body tissue (not fat) This loss of body tissue is a common finding in our patients with cirrhosis

We therefore feel that our patients need a high protein diet as well as a low sodium diet We agree with Dr Klatskin and with Dr Chalmers that if you put an alcoholic cirrhotic to bed on a minimal diet he will get much better, and we do this to all our patients to get them in a state of balance before we feed them high protein diets They will improve for twenty to thirty or sixty days and then they will level off If you feed more protein at that time they then will do very

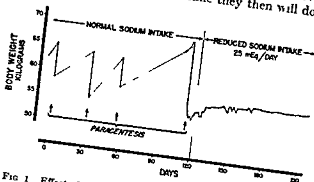


FIG 1 Effect of low sodium diet in preventing re accumulation of ascitic fluid

well Fig 2 shows a nitrogen balance during a 128 day's study of a patient who was eating about 180 grams of protein a day We kept his total water intake constant in a constant environment He is very much underweight He has no ascites He has a low sodium output, at a point early in the study and a high sodium output at the end of the study He has gained 13 kg weight He has gained 1,400 g nitrogen and if you multiply that by the protein factor of 32, you find that he should have gained about 43 kg bodyweight He has a low urine output in the first two weeks of the study,

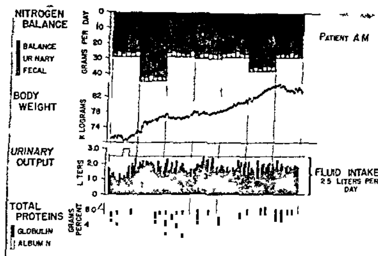


FIG 2 Nitrogen balance study of patient with cirrhosis on diet containing 180 g protein daily

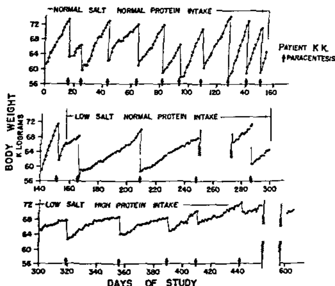


FIG 3 Long term study of weight changes in patient with cirrhosis on normal and low salt and protein intakes

and as he gets better his urine output increases, much greater than a normal control in the same sort of environment At the same time that he is laying down nitrogen his potassium balance is negative Therefore, we feel that a great number of cirrhotics have a chronic intracellular œdema, that is the have an excess of potassium and water, a potassium brine in their cells

Fig 3 is of a patient whom we studied in the metabolic ward for about 500 days You see numerous paracenteses

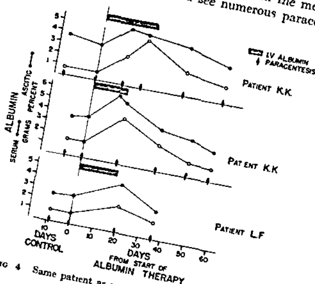


FIG 4 Same patient as in Fig 3 Infusion of albumin

when he is living on a normal salt, normal protein intake He cut down the salt to 33 milliequivalents per day and we low down the accumulation of ascites, but his dry body eight does not increase much We continue to keep his dium low but increased his normal protein intake to a high otein intake He immediately starts to gain body mass

There is no more ascites at the end of the study At three points early in the study, we infused albumin, and the albumin passed into his ascitic fluid At one point, at the end of the study, when we infused albumin, it did not pass into his ascitic fluid At a point at the very end of the study he has maintained his weight, he is perfectly fit, his liver function tests are

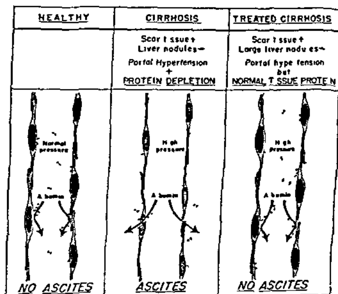


FIG 3 Mesenteric capillary diagram representing role of protein depletion in formation of ascitic fluid

quite normal but he has a large massive liver, full of big nodules and scar tissue and his portal pressure is high whether measured indirectly from abdominal collateral veins as Dr Davidson has done, or by measuring his hæmorrhoidal vein pressures

Fig 4 shows the passage of intravenous albumin into his ascitic fluid after infusion during eighteen days, with a total of 1,800 g of albumin

What we think happens in some patients is shown in Fig 5 Here you have a healthy individual with a healthy mesenteric capillary filled with normal tissue The pressure is normal The albumin cannot pass through the capillary wall If you tie the portal vein you raise the pressure but the albumin will still not go out into the peritoneal cavity Now a cirrhotic has portal hypertension and his tissues are generally protein depleted so that the capillary endothelial lining cells are depleted of protein Under these circumstances the albumin under high pressure will pass through the mesenteric capillaries into the peritoneal cavity Now if we take the same man in whom we know portal hypertension remains high and if we restore his normal tissue protein by high protein feeding we fill up these cells again The pressure is still high inside the vessel but infused albumin now does not pass through the capillary wall

DISCUSSION PORTAL HYPERTENSION IN CIRRHOSIS OF THE LIVER TREATED SURGICALLY BY PORTACAVAL SHUNT

ALLAN GAMMELTOFT

In cirrhosis of the liver deficiencies in the various liver functions, due mainly to atrophy of the parenchymal tissue, can be treated medically with benefit And the principle of the medical treatment must be to provide substitutes for the more vital functions and an attempt to reduce certain intermediary metabolic processes Treated by this principle over a long period, six to twelve months, the liver often regenerates to a great extent and will be able to function almost normally But the fibrosis in the liver once there, will never disappear, and portal hypertension, which is the result of fibrosis, will never decrease Nature cures or assists in curing this condition by the well known development of collateral circulation

With the dilatation of the collateral veins the portal blood by passes the liver, but the portal hypertension still persists And no medical treatment is able to diminish an already developed portal hypertension due to cirrhosis of the liver

In the cases where the collateral circulation makes its way through tributaries of the coronary vein to the œsophageal veins with the development of varicosities, then the indication for surgical treatment becomes obvious The evidence for this is confirmed by Patek's analysis of 124 cases of cirrhosis treated medically Forty four of these 124 cirrhotics had hæmatemesis, and within one year of the first bleeding episode, 22 of the cases died from hæmorrhage

During the past year I have had the opportunity to examine and follow up the cases of portal hypertension treated surgically by portacaval shunt operation performed by Blakemore from 1944 to date at the Presbyterian Hospital in New York

When I left the hospital 108 cases had been operated upon Seventy nine belonged to the group of cirrhosis of the liver Twenty nine cases were extrahepatic blocks with a typical Banti's syndrome, but without evidence of cirrhosis of the liver at the time of operation Twenty six of the 29 cases had either a cavernomatous transformation of the portal vein or an obstruction in the portal vein due to thrombosis Two cases had portal hypertension due to an arteriovenous fistula between the splenic artery and the splenic vein and one case had obstruction of the splenic vein because of a cyst in the pancreas

I present some illustrations showing the data of interest in the cirrhotic cases Table I shows the results of operation on cirrhotic cases up to 1st May, 1950 Sixteen cases died postoperatively and 12 cases died from six months up to four years after the operation. The next Table, No II, shows the ætiology of the cirrhosis in the surgically treated cases In Table III the major indication for surgical treatment is presented Hæmorrhage is by far the greatest, 59 cases out of 79 cases Ascites was the primary indication in ten cases though 15 additional cases showed some ascites present at operation Nine cases had general symptoms of cirrhosis and œsophageal varices shown by X ray but without bleeding episodes and one case presented Chiari's syndrome

Table I

CIRRHOSIS OF THE LIVER TREATED SURGICALLY BY PORTACAVAL SHUNT

	Males	*Females*	*Total*
Operated	42	37	79
Died Postoperatively	9	7	16
Died During Follow up	8	4	12
Alive 1/May/50	25	26	51

Table II

ÆTIOLOGY OF CIRRHOSIS IN SURGICALLY TREATED CASES (70)

Hepatitis	32	Schistosomiasis	2
Alcohol	11	Uncertain	16
Common Duct Obstruction	3	Various Hepato-Toxic Agents	6
Syphilis	6		

Table III

MAJOR INDICATION FOR SURGICAL TREATMENT IN CASES OF CIRRHOSIS

Hæmatemesis or Melæna	59
Ascites	10
Cirrhosis and Œsophageal Varices	9
Chiari s Syndrome	1

Table IV shows the type of portacaval shunt performed in the 79 cases The portal vein to vena cava, which is technic ally easier than the other types mainly because of the greater size and thicker wall of the vessels shows the lowest mortality, 16 2 per cent

Table IV

TYPE OF PORTACAVAL SHUNT IN SURGICALLY TREATED CASES OF CIRRHOSIS (79)

Portal Vein to Vena Cava	*37 (D ed Post op*	*6 (16 2 per cent)*
Splenic Vein to Renal Vein	39 (D ed Post op	8 (20 5 per cent)
Umbilical Vein to Vena Cava	1 (D ed Post op	1)
Portal Vein to Renal Vein	1 (D ed Post-op	1)
Inferior Mesenteric Vein to Vena Cava	1	

The causes of death during the follow up period in sur gically treated cases are recorded in Table V The 12 cases died from two months up to four years after the operation Three of the five cases which died from hæmatemesis and revealed a closed shunt at autopsy, had a splenorenal shunt performed by the vitallium tube technique The two other

Table V

CAUSES OF DEATH DURING FOLLOW UP IN SURGICALLY TREATED CASES OF CIRRHOSIS (12)

Hæmatemesis	with Closed Shunt	5
	with Open Shunt	1
Cholemia	with Closed Shunt	2
	with Open Shunt	1
Perforated Ulcer (no autopsy)		1
Hæmochromatosis with Open Shunt		1
Accidental (Pulmonary Œdema no autopsy)		1

cases with closed shunts had portal vein to vena cava anastomosis, one by vitallium tube technique and one by suture. The one case with an open shunt had a splenorenal anastomosis by suture. In the three cases which died from cholæmia, two showed closed shunts, one splenorenal and one portacaval, both by vitallium tube technique. The third case had an open shunt, a sutured portacaval anastomosis. The one case which died from hæmochromatosis had an open portacaval shunt performed by vitallium tube technique.

Even from this small number of cases it is obvious that the suture method is preferable to vitallium tube technique and also that the portal vein to vena cava is a better anastomosis than the splenic vein to renal vein.

Now, what about the late results?

Of 51 operated cases alive on 1st May, 1950, 38 cases have been operated upon more than one year ago. The oldest follow up case was operated in April, 1945. In Table VI the general condition of the 38 cases is shown. The perfectly well and the moderately well cases are all active, doing their jobs and have only minor complaints. The two poor cases in the male group are not able to work because of a duodenal ulcer and a subchronic abscess.

Table VI

GENERAL CONDITION OF FOLLOWED UP CASES OPERATED ON FROM ONE TO FIVE YEARS AGO (38)

	Perfectly Well	*Moderately Well*	*Poor*
Females (22 cases)	15	7	0
Males (16 cases)	10	4	2

The complaints in the moderately well group are shown in Table VII. The two cases with one mild bleeding episode was not admitted to hospital and detected the bleeding by accident. They are the only two cases also of the 51 cases alive on 1st May 1950, who had a bleeding episode post operatively. The cases with jaundice and ankle œdema are

improving with every follow up examination None of the cases have ascites

Table VII

COMPLAINTS IN THE MODERATELY WELL FOLLOWED UP CASES (11)

	One Episode of Mild Bleeding	Slight Jaundice	Slight Ankle Œdema
Females (7 cases)	1	2	6
Males (4 cases)	1	2	1

And how is the liver function in these patients? Table VIII shows the results of six important tests in the cases followed up for more than one year In the bromsulphthalein, A/G ratio, galactose removal constant, cholesterol test and the prothrombin time only a change of 10 per cent or more of the pre-operative level has been taken as improvement or the reverse For the cephalin flocculation test only a change of plus 2 or more has been regarded as significant All the cases have been proved to be cirrhosis of the liver by biopsy

Table VIII

THE INFLUENCE OF THE PORTACAVAL SHUNT OPERATION ON LIVER FUNCTION TESTS IN CASES OF CIRRHOSIS OF THE LIVER FOLLOWED UP FOR MORE THAN ONE YEAR AFTER OPERATION

	Improved	No Change	Worse	
Bromsulphthalein	8	20	4	(32)
Albumin/Globulin Ratio	6	20	11	(37)
Cephalin Flocculation	8	22	7	(37)
Galactose Removal Constant	4	2	5	(11)
Cholesterol Esters in per cent of Total	3	14	2	(19)
Prothrombin Time	3	12	1	(15)

The most important conclusion from these data is that only about 20 per cent of the surgically treated cases show progression of their liver damage due to cirrhosis, about 20 per cent show definite improvement and about 60 per cent show no change *Many of these latter cases have pre operative* values of liver function tests within the normal range And so, any improvement in these cases was impossible

Thus, in view of the relatively low operative mortality rate in comparison with the risk of death from hæmorrhage without operation, the relief of the repeated bleeding episodes and the minor harm of by passing portal blood from the liver, the portacaval shunt is an important aid in the treatment of ascites

In addition to these figures of the follow up results, I should like to present a Fig (No 1) which shows the portal

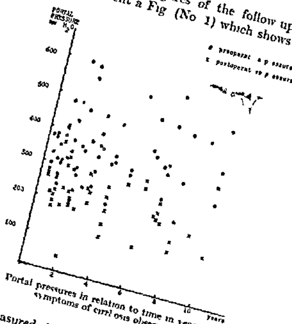


FIG 1 Portal pressures in relation to time in years since first symptoms of cirrhosis observed

pressures measured during the operations before and after the shunt has been established The values cannot be regarded as absolute pressures but they are all comparable lue to the measurement by the same method with a water ıanometer The average pre shunt pressure is approximately 00 mm H_2O and the average post shunt pressure approxi ately 200 mm H_2O The highest pre operative pressure

measured is 600 mm H_2O and the lowest post operative pressure measured is 40 mm H_2O

In Fig 1 the measurements of portal pressures are plotted in relation to the time in years which elapsed from the observation of the first symptom of cirrhosis of the liver up to the time of operation The longest period has been ten years From this figure it is obvious that there is no tendency to increase or decrease of the portal pressures during the years of history If an increase in the portal pressure takes place during the years of cirrhosis of the liver a line drawn from the average pressure values will show a rise from left to right according to the time elapsed from the first symptom up to the operation This Fig (No 1) shows very definitely that the average pressure line drawn from the dots is very near to the horizontal line and parallel to the abscissa

The observations above described would indicate that when the cirrhosis shows definite symptoms then the portal hyper tension is maximal and neither increases or decreases during the disease And from these findings one would conclude that a patient is better off with a portacaval shunt operation early in the disease than later when the liver function is badly damaged

I would like to make a few comments on ascites as an indica tion for portacaval shunt operation Ten of the 79 cases were treated surgically for portal hypertension with ascites as a major indication None of the 38 cases followed up for more than one year showed any sign of ascites

From what we heard to day portal hypertension is only one of many factors in producing ascites Ascites is only a partial indication for the performance of a portacaval shunt in contrast to œsophageal varices as an absolute indication

Only after an attempt to treat and control some of the other factors the colloid-osmotic pressure and the sodium retention then the performance of a portacaval shunt to reduce the portal hypertension can be discussed From animal experiments it is shown that stasis in the lymphatics an increased production of the antidiuretic factor and VDM

substance are also responsible for the production of ascites But any intelligible treatment of these three factors is not known at the moment

An interesting observation was the production of post-operative ascites in the cases operated upon for œsophageal varices, which did not have any sign of fluid in the abdomen pre operatively The explanation of this is probably the heavy loading with sodium during the operation where 2,000–4 000 ml of blood in sodium citrate was transfused Between 5 and 10 g of sodium was given by this route Treated with a diet rich in protein, human albumen intravenously and a salt free diet, the ascites disappeared after one to three months

DISCUSSION · CIRCULATORY STUDIES ON PATIENTS WITH ASCITES

SHEILA SHERLOCK

I should like to show you some circulatory studies which)r Sheila Howarth and I made at the Postgraduate Medical chool on patients with tense ascites from various causes

Pressures were estimated with a saline manometer in the eritoneal cavity and in the right auricle, using cardiac atheterization In the patient illustrated (Fig 1), the intra

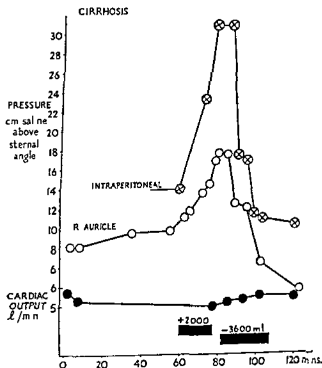


FIG 1 The effect of ascites on intraperitoneal and right auricular pressures and on cardiac output Introduction of more fluid into the peritoneal cavity is marked + and paracentesis —

peritoneal pressure was very high and, as is often found clinically with ascites, the jugular venous pressure was also raised above normal The latter was reflected as one would anticipate, in a high right auricular pressure It is the mechanism of this raised right auricular pressure that I wanted to discuss Cardiac output was also estimated At sixty minutes, two more litres of fluid were introduced into the peritoneal cavity, resulting in a great increase in intra peritoneal pressure The right auricular pressure also rose,

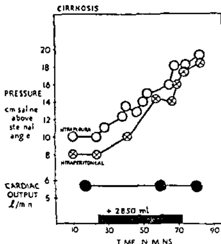


Fig 2 The effect of ascites on intraperitoneal and intrapleural pressures and on cardiac output Introduction of more fluid into the peritoneal cavity is marked +

but cardiac output was unaltered The fluid was now let off (82 minutes) and there was a fall in intraperitoneal pressure and in the right auricular pressure Again the cardiac output was unaltered This, then is an example in which the right auricular pressure has been greatly altered by the conditions below the diaphragm, but in which the cardiac output did not change

In a patient with pleural effusion and ascites we also measured intrapleural pressures (Fig 2) Again we raised the pressure in the abdomen by putting in more fluid We caused a rise in the intraperitoneal pressure and in the intra pleural pressure, but again the cardiac output didn't change We believe the raised right auricular pressure and the raised venous pressure in the neck occurring in patients with ascites are merely secondary to the raised diaphragm and raised intrapleural pressure resulting from the increased tension in the abdomen

DISCUSSION ELECTROPHORESIS IN HEPATITIS, LIVER ATROPHY AND CIRRHOSIS

G VIOLLIER

I would like to mention a few electrophoresis results obtained from cases of hepatitis observed during the epidemic of 1946 in Basel The data may be of value in furthering the relationship between hypoproteinæmia and ascites and œdema development in chronic liver diseases discussed earlier by Dr Iversen We were surprised to note an almost normal A/G quotient in many cases of acute and sub acute liver atrophy Similar A/G quotients were observed in severe hepatitis with pronounced hypoproteinæmia ascites and œdema One could perhaps assume that the physio logical function of the albumin may have been altered through the injury of the liver although there is no experi mental evidence for this assumption especially not on physico chemical grounds Other cases without ascites and œdema dying of subacute liver atrophy having a normal serum protein content had a quotient well below 1 It may be that in these cases the period of illness was too short and too acute for the establishment of severe hypoproteinæmia and the consequent development of œdema and ascites Generalizing we can say that during the epidemic of 1946 ascites and œdema appeared only when hypoproteinæmia had been present for several weeks beforehand

Material and Method

The material at our disposal was in its way unique There were in the year 1946 in the whole town of Basel 220 cases of hepatitis of which 44 proved fatal a mortality of 20 per cent Such a high death rate due to hepatitis has perhaps never been recorded in any one such small community and

over such a short period of time One hundred and forty nine cases were admitted into the Burgerspital from February 1946 to April 1947 Forty one of the admitted patients died, 30 of them in the months of June, July and August, 1946 Thirteen showed severe hepatic failure but recovered after long treatment, while in 95 cases the hepatitis was of the common benign type Prof Staub has reported on clinical observations in previous papers (Staub, 1946*a* and *b*, Staub 1947) Table I shows how the peak death rate of 1946 gradually decreased till it came back to the usual level again between the years 1948 50

Table I

MORTALITY OF THE HEPATITIS EPIDEMIC IN BASEL

Year	Number of cases	Fatal cases	Mortality in per cent
1942	315	3	0 96
1943	703	2	0 28
1944	244	1	0 41
1945	87	1	1 15
1946	220	44	20 00
1947	79	7	8 90
1948	89	4	4 50
1949	101	1	0 99
1950	38	—	—

Changes in serum protein are not, strictly speaking specific for liver impairment but the electrophoresis analyses still give the best information on the function of serum proteins in liver diseases This method does not merely fractionate the serum proteins into their various physico chemical parts but at the same time is a means of separating the specific physiological functions carried by each component In 1946/47 we performed electrophoresis analyses using Tiselius' original cell 45 mm in height, and a barbiturate acetate buffer of *p*H 7 8 Since 1948 we have used Longsworth's cell, 90 mm in height, and his diethylbarbiturate buffer, having a *p*H of 8 5 However, by 1948, there were no

longer many cases of liver atrophy, so I am afraid that the results I wish to mention are those carried out under the old experimental conditions Some have already been published (Wolher and Staub, 1949), a few more typical cases are to be presented now

Results

In contrast to benign hepatitis, where the β peak is enlarged, serum from liver atrophy patients shows a small β and pronounced γ peak The whole curve appears flattened and drawn out, due to the formation of a platform between the β and γ-globulins This platform we presumed to be caused by an unknown globulin component which we called H from hepar Dr Popper pointed out to me that this component has been described by others as a γ_1 fraction I should prefer to call it H as this globulin is not present in normal sera We have found H in sera obtained from cases of idvanced cirrhosis of the liver rheumatic polyarthritis, Iodgkin s disease and obstructive jaundice Whereas in dvanced cirrhosis and liver atrophy this component may nch a level of from 6–10 per cent of the total protein, it mains in the other cases below 6 Even though the H nponent may not be of great value diagnostically, we feel at for prognosis an H level of between 6 and 10 per cent ...a be regarded as fatal

Another most striking fact we observed was the continuous fall in albumin concentration in cases of chronic liver atrophy developing into cirrhosis while in benign hepatitis the albumin concentration falls less and rises back to normal during recovery The serum esterase shows a parallel behaviour The determination of this enzyme activity is, as Dr Kark has mentioned a further important test useful in managing hepatitis

The Takata reaction, too was found to be useful during this epidemic in recognizing cases of severe liver parenchyma impairment This reaction introduced by Prof Staub in 1928 as a liver function test (Staub 1929 Staub and Jezler,

1935), has been found to be positive in cases where the γ-globulin concentration is high This is especially true when the albumin is also reduced In patients, therefore, where these protein conditions are realized and yet no liver damage is present the Takata can be positive, while in a few cases of obvious liver injury the Takata reaction may be negative From experience gathered in 1946–47, severe liver damage usually resulted in a positive reaction which with progressing severity of the disease became more and more strongly positive On all accounts a constantly positive Takata reaction in jaundice means serious parenchymal injury As the patient recovers a strongly positive Takata will gradually become negative again This may suffice to show the useful ness of the Takata reaction as a liver function test

Finally, the presence of low sedimentation rates and small fibrinogen concentrations were found to be valuable, if not sure signs of threatening liver atrophy In those cases where the sedimentation rate and fibrinogen concentration increased there was recovery

Here then are some examples where the course of the disease was followed by electrophoretic analyses

The first case (Fig 1), a woman, 47 years old P B, having suffered for several years from a gastric ulcer is an example of *subacute atrophy* of the liver A month before admission to hospital the urine became dark in colour, followed a fort night later by jaundice On examination (beginning of August, 1946) the Takata was slightly positive and the total serum proteins nearly normal The electrophoresis showed a slight increase in γ globulin and an A/G quotient of 0 93 The only disturbing facts were the low sedimentation rate and the small fibrinogen concentration (156 mg per cent) During clinical observation the A/G quotient fell consistently and on the 19th of August the Takata reaction was strongly positive Despite numerous blood and plasma transfusions, diuresis was not raised, and there was much vomiting At the beginning of September a marked decrease in liver volume was noted followed by fœtor hepaticus, mental confusion

and finally coma The γ globulin increased up to death There was no ascites, total protein 7 23 g per cent Fibrinogen concentration and sedimentation rate were constantly low At autopsy the liver was small and atrophied, weighing 730 g, the left and right lobes were almost completely collapsed with a few parenchymal remains showing adenoma

Bilirub mg%	Fibrinogen mg%	Sediment rate mm	Proteins total g%	Takata 20°	Electrophoresis: Diagr. of desc. bound	Albumin %	Globulines % α	β	H	γ	Quot A/G	Ascites Oedema	Weight Kg
3,9	156	5/13	6,59		2,8 46	48,3	5,2	17,1	–	29,4	0,93		60,4
3,2	250	5/12	6,34		10,8 46	49,1	3,9	16,0	–	31,0	0,98		61,1
8,2	194	7/9	6,19		19,8 46	42,4	3,8	18,5	–	35,5	0,74		61,3
–									–				57,8

FIG 1 Subacute liver atrophy leading to death two months after the development of jaundice

arrangement, severe, widespread jaundice, cholæmic hæmorrhage and nephrosis, gastric ulcer

In *subchronic atrophy* the course of the disease is slower, with development of ascites and œdema Such was the case in a young girl of 22 years, St A (Fig 2) who came into hospital six weeks after onset of jaundice On admission the patient already showed signs of ascites and œdema, reduced liver volume, pronounced hypoproteinæmia, slightly positive

Takata reaction, little fibrinogen and low sedimentation rate. The electrophoresis analyses gave an A/G quotient of 1.16. Daily plasma transfusions caused a temporary increase in

Bili rub mg%	Fibrinogen mg%	Sediment rate mm	Proteins g%	Takata 20°	Diagr of desc bound	Albumin %	Electrophoresis Globulines % α	β	H	γ	Quot A/G	Ascites and Oedemas	Weight kg
63	188	6/17	5,58		31 7 46	53,1	5,6	9,8	3,7	27,9	1,16	2,9 +	68,1
59	119	4/17	5,47		21 8 46	53,9	4,7	11,7	4,2	25,5	1,17	5,5 +	70,0
5,35	163	2/8	5,16		7 9 46	54,5	5,5	11,5	2,9	25,6	1,20	10,7 +	68,5
5,5	213	18/31	5,68		25 9 46	51,0	6,2	11,7	4,9	26,2	1,04	7,4 +	59,0
7,45	169	2/3	5,12		11 10 46	42,9	7,5	8,1	6,7	34,8	0,75	32,5 +	56,5
6,50	219	15/37	5,25		25 1[illegible] 6 46	[illegible]	[illegible]	[illegible]	8,0	30,7	0,79	+	[illegible]

St. A 22 years ♀

Subchronic liver a... development of ... a column refer... drawn at th... to death f... figures in... in litres of... rvals

asci... ct... d...

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ser... reni...

weight, but the hypoproteinæmia was not improved The electrophoresis pattern followed the increasing severity of the patient's condition. the A/G quotient steadily became lower, the γ-globulin increased and the H-component, which was 3 7 per cent at the beginning, rose to 8 0 per cent Closely following these alterations the Takata reactions

Bilirub. mg%	Fibrinogen mg%	Sediment rate mm	Proteins g%	Takata 20°	Diagr of descbound	Albumin %	Globulines % α	β	H	γ	Quot A/G	Ascites and Oedemas	Weight kg
4,45	200	42/70	8,41		10 Q 46	39,7	6,8	8,8	4,1	40,8	0,66	+	45,2
norm	381	51/86	6,91		30. 7 46.	50,8	9,5	13,7	–	26,0	1,03	(+)	40,2
norm	41,9	32/66	7,69		8 47	50,4	7,5	19,3	–	22,8	1,01		40,4
0,64	400	56/96	6,95		20 1 47	53,1	6,7	18,7	–	23,5	1,14		41,0

St.R 50 years ♀

FIG 3 Severe hepatitis with ascites and œdema recovering four months after the development of jaundice

became more and more strongly positive The patient died in coma hepaticum, four months after the onset of jaundice, the fibrinogen content and sedimentation rate having remained constantly low

Fig 3 shows the return of the electrophoretic pattern to normal in a case of *hepatitis with severe hepatic failure* The jaundice in the patient, a 50 year old woman, St R , started about two weeks before entry into hospital Examination

showed the presence of ascites and œdema, slight hypoproteinæmia and positive Takata reaction. The A/G quotient was 0.66, the H-component 4 1 per cent of the total protein

Bil rub mg%	Fibrinogen mg%	Sedimentation rate mm	Proteins g%	Takata 20°	Diag. of desc. bound (Electrophoresis)	Albumin %	Globulines % α	β	H	γ	Quot. A/G	Asc. and Oedemas	Weight kg
4,56	362	23/46	5,90		27 8 48	37,8	7,9	17,0	4,3	33,0	0,71	+	67,2
5,10	325	55/78	6,90		8 7 48	40,2	6,2	20,1	–	33,5	0,68	(+)	55,2
6,36	338	72/94	7,49		27 7 46	42,8	7,2	16,8	–	33,2	0,77		52,2
4,75	400	90/118	6,86		8 8 48	48,5	8,0	13,9	–	31,6	0,87		51,8
1,90	388	42/71	6,95		9 9 48	45,3	6,7	14,2	3,1	30,7	0,82		50,8
1,58	300	20/43	6,01		0 10 48	49,6	6,9	14,1	–	29,4	0,99		52,0

R.F. 46 years ♀

FIG 4 Severe hepatitis with liver atrophy, recovering five months after the beginning of jaundice

content Further clinical findings hypertonia with slight myocardial damage The liver was enlarged Treatment strophoside and repeated plasma transfusions Recovery followed with increase in albumin and fall in γ-globulin

concentrations, and the disappearance of the H component At discharge, four months after the beginning of jaundice, the Takata reaction was however still strongly positive The liver had returned to the normal size The patient had a high sedimentation rate throughout which is characteristic of cases progressing favourably

A similar case is shown in Fig 4 The patient, a 46 year old woman, R F, developed jaundice three weeks before admission to hospital She was apathetic on admission The liver was markedly reduced, there was repeated vomiting and ascites and œdema were present Laboratory tests showed a pronounced hypoproteinæmia, Takata reaction positive, A/G quotient 0 71 and the H component 4 3 per cent However, the fibrinogen concentration and sedimentation rate were both fairly high With repeated plasma transfusions the total serum protein concentration rose, diuresis increased and the ascites and œdema disappeared Recovery was slow, the albumin concentration rose very slowly, the A/G quotient reaching a level of 0 99 after four months The Takata on discharge, however, was still strongly positive, and the liver enlarged with a hard edge

In comparison with these severe cases Fig 5 shows the usual course of two cases of *mild hepatitis* In the first patient, a 42 year old woman W M a few days after admission, the bilirubinæmia reached 9 95 mg per cent the Takata was strongly positive and there was a passing hypoproteinæmia The β globulin concentration was high Soon there followed the beginning of recovery with increase in the A/G quotient When the patient left, 34 days after the outbreak of jaundice, the Takata was only weakly positive By studying the electrophoretic patterns one can follow the reduction of the relatively high β globulin concentration

The second case a 40 year old woman G M, had a similar history On the 17th March 1947 (two weeks after admission), the bilirubin concentration rose up to 9 85 mg per cent With recovery the usual rise in albumin concentration and fall in the β globulin peak were observed On discharge on

the 7th July the Takata was completely negative High fibrinogen and sedimentation rate were noted throughout

There is a discrepancy between the chemically and electro phoretically determined albumin concentrations (see Fig 5)

Bilirub mg%	Fibrinogen mg%	Sediment rate mm	Proteins g%	Takata 20°	Diagr of desc. bound.	Electrophoresis: Albumin %	Electrophoresis: Globulines % α	β	φ	γ	Electrophoresis: Quot A/G	Chem Analysis: Albumin %	Chem Analysis: Globulines %	Chem Analysis: Quot A/G	Weight kg
0,95	281	14/31	6,17		14 12 46	50,7	7,1	19,2	—	23,0	1,03	55,1	44,9	1,23	53,9
0,88	294	19/4,5	7,62		6 1 47	53,7	8,9	15,0	—	22,4	1,16	61,7	38,3	1,61	52,4
—	300	23/50	7,56		6 2 47	54,2	9,1	11,8	—	25,0	1,18	58,5	41,5	1,41	53,7
WM 42 years ♀															
0,18	306	25/54	7,43		3 3 47	45,6	5,9	21,8	—	28,7	0,84	50,9	49,1	1,03	78,9
0,74	413	68/107	7,01		14 4 7	48,8	7,8	17,0	—	26,5	0,96	53,2	46,8	1,13	77,0
—	431	65/100	7,86		5 5 47	52,0	10,9	14,3		22,9	1,08	59,3	40,7	1,45	78,8
GM 40 years ♀															

FIG 5 Two cases of benign hepatitis showing a comparison between electrophoretically and chemically determined A/G quotient

But due to its convenience and rapidity the salting out method is widely used *The chemical A/G quotient is some what higher than that determined electrophoretically, although its trend on recovery of the patient, is practically identical with the other*

In Fig 6 there are two examples of recent cases examined with the improved electrophoresis technique (Longsworth's cell and barbiturate buffer pH 8 6) Under these conditions

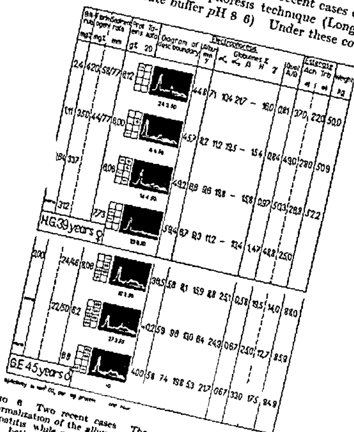


FIG 6 Two recent cases The upper diagram shows the normalization of the albumin and esterase in a case of benign hepatitis while in the lower a case of liver cirrhosis although both rise in concentration they do not reach normal

α_1 globulin separated quite clearly from the albumin and the ϵ boundary from the γ globulin In these tables are included

results of serum esterase determinations Acetyl choline iodide and tributyrine were used as a substrate in a medium of Krebs' bicarbonate Ringer solution *The evolved carbon* dioxide was determined manometrically Activity is given in mm ^{3} CO_2 per mg serum protein an hour In the first patient, a 39 year old woman, H G, having a hepatitis of the benign type, one sees on recovery the increase in the albumin and the parallel reduction in β and γ globulin peaks The esterase activity also increases till it reaches normal

The second case, a 45 year old male, G E with cirrhosis of the liver and delirium tremens shows a similar rise in albumin and esterase activity on improvement In contrast to the first the values do not come back to normal so that there is only improvement in the patient's condition not complete recovery The H component receded from 8 8 to 5 8 per cent the γ globulin still remained high, characteristic of liver cirrhosis The Takata was strongly positive throughout and at discharge the liver was enlarged with a hard edge

Conclusion

From experience we find that the electrophoretic study of the variation in serum proteins, in liver injury, is of great prognostic value *In hepatitis ascites and œdema may* develop even with a normal A/G quotient if there is a constant hypoproteinæmia and the jaundice is of long standing We have examined cases with ascites and œdema in which the electrophoretically determined serum albumin was high with the γ globulin but slightly increased e g 62 per cent albumin and an A/G quotient of 1 63 The results of repeated electrophoreses in such cases were closely parallel with the clinical observations In recuperating patients the electrophoresis diagram remained unaltered while in cases where the disease led to subchronic liver atrophy (see Fig 2) the albumin fell and the gamma globulin rose constantly until finally

the electrophoretic pattern was almost indistinguishable from that obtained in cases of cirrhosis

Summing up we found the following protein changes important in determining the damage caused by hepatitis to liver cells —

1 A fall in albumin concentration This was found with great regularity in both serious and mild cases With equal regularity the albumin came back to normal as the patient recovered, while it continued to fall if the case got worse Without doubt these observations help to support, on a clinical basis, the generally accepted opinion that albumin is formed in the liver From experiments on animals with liver damage there appears to be no definite proof supporting this opinion (Cheng, 1949) In our electrophoresis experiments with serum from rats suffering from prolonged choline deficiency or DMAB hepatomas, we have found no significant albumin reduction (Violier, 1949, 1950) The albumin concentration was, however, markedly reduced in rats having subcutaneous tumours (induced by methylcholanthrene and benzpyrene)

2 A high β peak with a moderate γ concentration is typical of mild hepatitis The β peak sinks back to normal during convalescence while the γ concentration remains high for a little longer This also explains why the Takata reaction may still be positive even after most of the clinical signs have disappeared

3 It is difficult to explain the appearance of the H component and the rise in γ globulin concentration when the hepatitis develops into liver atrophy or cirrhosis Were this change a compensation for the loss in albumin concentration, then the α and β globulin concentrations would have to change proportionally to the γ But we have seen that the γ globulin can rise independently in concentration till the patient dies Dr Bjorneboe has suggested that this may be due to an increase in antibodies We cannot check this statement with the material at hand The increase in γ globulin in sub acute liver atrophy we have observed often,

showing a similar picture to that seen in cirrhosis although there is no trace of increased connective tissue Yet parallel to the increase in γ globulin, we find an infiltration of lympho cytes into the peri portal spaces (this may be the "pre cirrhotic state") Another explanation offered by Dr Martin (Martin, 1949) is that the γ globulin may normally be destroyed in the liver for its subsequent excretion or rebuilding The diseased liver would, due to its impairment, be incapable of continuing this function This would explain the enormous rise in γ globulin in liver atrophy and cirrhosis, though I have not yet found any experimental support for this hypothesis This theory being acceptable one would have to postulate the extra hepatic formation of γ globulin Our electrophoresis analyses in cases of liver atrophy and cirrhosis do not in any way hinder this assumption

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GENERAL DISCUSSION

W VOLWILER I am very glad that both Professor McMichael and

escape from the capillary bed into free tissue spaces It seems to me that the lymphatic circuit is exactly analogous, and that along the way towards the thoracic duct, fluid can likewise escape from the lymphatic channels

Professor Best has asked me to make a few more remarks about the anatomy of the lymphatic circulation of the liver, particularly the human liver I don't believe we know much about the lymphatic

efferent channels that one sees along the portal vein one can also

thorax There remains a great deal of work to be done on the anatomy

I would like to ask Mr Macpherson what he thinks is the normal portal vein pressure in man

H G KUNKEL I certainly agree with Professor McMichael that

showing a similar picture to that seen in cirrhosis although there is no trace of increased connective tissue Yet parallel to the increase in γ globulin we find an infiltration of lymphocytes into the peri portal spaces (this may be the pre cirrhotic state) Another explanation offered by Dr Martin (Martin 1949) is that the γ globulin may normally be destroyed in the liver for its subsequent excretion or rebuilding The diseased liver would due to its impairment be incapable of continuing this function This would explain the enormous rise in γ-globulin in liver atrophy and cirrhosis though I have not yet found any experimental support for this hypothesis This theory being acceptable one would have to postulate the extra hepatic formation of γ globulin Our electrophoresis analyses in cases of liver atrophy and cirrhosis do not in any way hinder this assumption

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GENERAL DISCUSSION

[illegible] I am very glad that both Professor McMichael and

in Dr Sherlock s observation that the right auricular pressure seemed to reflect to some extent the pressure in the abdomen Without doing auricular pressures I wasn t able to find any correlation at all between the drainage into the superior vena cava and the portal pressure There is a close correlation between the intra abdominal pressure and the pressure in the leg veins

the post necrotic type of cirrhosis because the case appeared to be less *complex than the average case of Laennec's cirrhosis with marked ascites* In this patient two factors appeared to be dominant, hypo

, and we were able to get

played

lium excretion in control

A I S MACPHERSON Professor McMichael mentioned that the

recent paper from the Mayo Clinic He also said that hæmorrhage in portal hypertension is not necessarily from œsophageal varices Again I entirely agree with him Operation must never be undertaken unless hæmorrhage can be proven to be from œsophageal varices

I was most interested in Dr Gammeltoft s continuation of some of the work that I had been doing in the States with Dr Blakemore and particularly in the fact that he was able to demonstrate that 20 per cent of the cases showed improvement in liver function after operation But I don t think he will be so bold as to ascribe that improvement to the operation It does I think, reflect that the liver is now able to work in a much better environment, it is not losing either blood protein or plasma protein and its nutrition is better When I was speaking about ascites, I described those cases in which the ascites was persistent and was associated with obvious signs of liver damage Dr Gammeltoft and I were at cross purposes there because I deliberately avoided including any in which the ascites could be associated with severe hæmorrhage immediately preceding it

Dr Volwiler asked me about the normal portal pressure I did a series of cases in people who were being operated on for various abdominal complaints other than cirrhosis In patients anæsthetized

anæsthesia or with the presence of cyanosis due to obstructed respira tion You could increase the venous pressure in the arm very greatly without affecting the portal pressure at all Therefore I was interested

fæcal stercobilin, this stercobilin cannot be derived from hæmoglobin disintegration, as the stercobilin derived from the labelled hæm first appeared as a second wave in the fæces about 120 days later, corresponding to the lifetime of the erythrocyte Rittenberg estimates that about 15 per cent of the bilirubin is formed by synthesis and the rest from hæm under normal conditions, but that the synthetic fraction may increase to 50 per cent in certain blood diseases Gray, Neuberger and Snéath confirmed these observations but felt that the initial rise in the heavy nitrogen in the stercobilin might be due to disintegration of erythrocytes within the bone marrow before they reach the circulation

Thus bilirubin may be formed from other sources than hæmoglobin, and hæmoglobin may give rise to formation of other pyrrole compounds than bilirubin—but to what extent and influenced by which factors these processes, so contradictory to our customary ideas, really take place, is not known But it is clear that one cannot base calculations of the amount of hæmoglobin broken down on the amount of bile pigments excreted

That the liver is capable of disposing of about twenty times as much bilirubin as is produced under normal conditions has been generally assumed—a view advanced by Rich and mainly based on McMaster s experiments with ligation of increasing proportions of the bile ducts in dogs and monkeys But this view can hardly be maintained after Bollman and Mann demonstrated hyperbilirubinæmia in dogs after removal of two thirds of the liver, and Thompson and Wyatt, and Hench observed progressive bilirubin retention in humans after daily intravenous injections of bilirubin in quantities corresponding to twice the normal bilirubin production After this, a reduction of the excretion capacity to a third or an increase of the production to three times the normal should be enough to cause hyperbilirubinæmia

The so called direct diazo reaction of Hijmans van den Bergh has been much discussed In my opinion it is a curious biochemical phenomenon with limited interest The reasons

PART IV

PIGMENT METABOLISM

Chairman C J WATSON

BILE PIGMENT METABOLISM

A Brief Survey of Problems and Recent Developments

TORBEN K WITH

BILE pigment metabolism has turned out to be a much more complicated matter than was generally assumed This is clearly shown by the recent monograph of Lemberg and Legge who deal admirably with many aspects of bile pigment metabolism and propose a simplified nomenclature for bile pigments which is strongly recommended for use in future publications to counteract confusion

Hæmoglobin is broken down to bilirubin but not all its hæm gives rise to bilirubin but may give dipyrrole compounds such as bilifuscin which in certain cases may dominate over bilirubin That hæmoglobin may be broken down to compounds other than bilirubin makes it possible to understand the rare cases of biliary obstruction and acute yellow atrophy without jaundice which have been known for several years but were impossible to explain On the other hand it can no longer be taken for granted that all bilirubin is derived from disintegration of hæmoglobin as part of it is formed by direct synthesis as a by product of the protoporphyrin synthesis leading to hæmoglobin This is known from feeding glycine labelled with heavy nitrogen (Rittenberg and col laborators) which was rapidly followed by incorporation of the heavy nitrogen in the circulating hæmoglobin and about equally as rapidly by the appearance of heavy nitrogen in the

fæcal stercobilin; this stercobilin cannot be derived from hæmoglobin disintegration, as the stercobilin derived from the labelled hæm first appeared as a second wave in the fæces about 120 days later, corresponding to the lifetime of the erythrocyte Rittenberg estimates that about 15 per cent of the bilirubin is formed by synthesis and the rest from hæm under normal conditions, but that the synthetic fraction may increase to 50 per cent in certain blood diseases Gray, Neuberger and Sneath confirmed these observations but felt that the initial rise in the heavy nitrogen in the stercobilin might be due to disintegration of erythrocytes within the bone marrow before they reach the circulation.

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The so-called direct diazo reaction of Hijmans van den Bergh has been much discussed In my opinion it is a curious biochemical phenomenon with limited interest. The reasons

for this view are given in a monograph Biology of Bile Pigments which I hope to finish shortly and its section dealing with the biological significance of the direct reaction you will find in an appendix to this paper

The limits of normal serum bilirubin are not definitely known In adult man values below 1 mg per cent are considered normal but the upper limit is difficult to determine and the lower limit has not been discussed because the ana lytical methods in general use cannot determine low values with sufficient accuracy But methods fit for this purpose exist The fixation of the upper limit is difficult because of the existence of the condition called hereditary non hæmolytic hyperbilirubinæmia which is difficult to classify—some cases may be extreme variants of the normal some may represent a metabolic anomaly and according to Hult many, perhaps most cases generally placed in this group are really posthepatitic syndromes after abortive cases of viral hepatitis Hult based this conclusion on his finding of the same type of fatty infiltration of the liver in posthepatitic syndromes and his cases of non hæmolytic hyperbilirubinæmia

The site of the bilirubin formation has been much discussed Most writers feel that bilirubin can only be formed in the reticulo endothelial system and others that it is mainly formed in the parenchyma cells of the liver Lepehne (1930) proposed the dualistic theory according to which it is formed in both This theory has been criticized because bilirubin formation from hæmoglobin was looked upon as such a specific function that it could not be ascribed to two types of cells so fundamentally different But since Lemberg has shown that hæmoglobin breakdown cannot be considered a specific function this objection cannot be regarded as valid It seems most likely that bilirubin formation takes place in *all* tissues but under normal conditions mainly in the reticulo endothelial system

The fate of bile pigments formed in the organism has been subject to changing views Lemberg holds that not bilirubin but biliverdin is the compound primarily *formed from*

hæmoglobin in the organism and that biliverdin is reduced to bilirubin in the tissues According to Baumgartel this reduction may be carried further to mesobilirubin and mesobilane (urobilinogen IXa) in the tissues and in the bile and is caused by a special enzyme formed by the cells of the reticulo endothelial system This enzyme does not occur in herbivorous animals for which reason their bile is green

Whether bilirubin is absorbed from the intestine has been much discussed and is not known with certainty Because molecular bilirubin solutions do not pass collodion membranes (Bungenberg de Jong) and coproporphyrin is known not to be absorbed absorption of bilirubin cannot be regarded as likely Bilirubin does not undergo change in the small intestine, but in the colon it is hydrated to tetrahydromeso bilane (stercobilinogen) by bacterial enzymes as has been shown in *in vitro* experiments by Baumgartel This takes place throughout the colon by means of the dehydrogenase from dead *Bacillus coli* and the necessary hydrogen is delivered by an oxy reductive system consisting of the amino

bilane is formed in this process He further holds that mesobilane is never found in fæces but this is certainly wrong But when he believes that tetrahydromesobilane can never be formed from mesobilane by the action of bacterial enzymes he may be right The most likely explanation of the urobili noid formation at present seems to be that tetrahydromeso bilane is only formed—directly without intermediary products—in the intestine by bacterial enzymatic hydration of bili rubin and that mesobilane can be formed both outside and in the intestine by other processes than those giving rise to the formation of tetrahydromesobilane The role of the intestinal flora is most clearly shown by the recent experiments of Sborov and Watson who sterilized the intestine with aureo mycin after which the urobilinoids disappeared from the fæces and bilirubin itself took their place

After the investigations of McMaster and collaborators it was generally accepted that urobilinoids were only formed in the intestines, until German investigators pointed out, about ten years ago, that urinary excretion of urobilinoids in acute hæmolytic conditions begins so soon, that the bilirubin formed by the hæmolysis cannot have reached the colon and been transformed into urobilinoids with such speed Hence it was *necessary to assume extra intestinal formation in hæmolytic* conditions Baumgartel feels that mesobilane is regularly formed extra intestinally, but curiously enough he finds only increased excretion of tetrahydromesobilane and not of mesobilane in hæmolytic conditions He has advanced many interesting theories, but is often hard to follow, and his experiments and observations have mainly been published as brief summaries He has just published a monograph "*Physiologie und Pathologie des Bilirubinstoffwechsels als Grundlagen der Ikterusforschung*" (Georg Thieme, Publisher, Stuttgart)

Much work is required before the laws will be known which govern the occurrence in urine and fæces of mesobilane (urobilinogen) and tetrahydromesobilane (stercobilinogen), and it is not yet known whether it be of clinical importance to distinguish them from each other, a distinction possible by means of the ferric chloride mesobiliviolin reaction of Lem berg As the well known classical theory explaining the occurrence of urobilinuria in liver disease is based on exclu sively enterogenous formation of urobilinoids, it needs revision However, as there is generally good agreement between clinical findings and theoretical expectations, the *enterogenous formation* of urobilinoids is probably the more important in diseases of the liver and bile passages

Dealing with urinary bilirubin, it is important to remember that all bilirubin in serum is always firmly attached to the serum albumin (cf With 1948) Hence it cannot filter through the glomeruli and can only be excreted through the kidneys by active tubular secretion *The bilirubin clearance* of the kidneys is very low, 0 1 0 5 ml per min *In contrast*

to this the secretory capacity of the liver is enormous The serum threshold for renal excretion shows considerable individual variation, and is probably different in increasing and diminishing jaundice, and is certainly much higher in hæmolytic jaundice and icterus neonatorum than in other forms of jaundice This has been ascribed to the direct-indirect reaction of the serum bilirubin, but, although some connection exists, it cannot be a simple one, and it is certainly an over simplification to postulate that only bilirubin with the direct reaction appears in the urine This is perhaps most clearly shown by the experiments of Mann *et al* (1924) who found well marked bilirubinuria in their dehepatized dogs, in whom the serum bilirubin showed indirect reaction

Several investigators have demonstrated bilirubin in normal urine, and I have confirmed this in 70 out of 100 normal urines by filtering about 50 ml through talc and performing the Harrison and diazo tests on the coloured surface of the talc This is of interest, as slight bilirubinuria has been used as a sign of early viral hepatitis

The excretion of urobilinoids through the urine is governed by other laws than that of bilirubin, they occur only in very small quantities in serum 0 5 mg per cent at highest, and are excreted rather easily with both urine and bile

studied especially by von Dobeneck, are formed in urine and bile from bilirubin and urobilinoids and are very labile and impossible to determine quantitatively They are perhaps

strong tendency to polymerization Mesobilifuscin occurs regularly in the fæces as the chromogen pre mesobilifuscin which may be found in larger amounts than the urobilinoids, according to Siedel These substances cannot be determined

quantitatively, as they are labile and do not give characteristic colour reactions

The theory of jaundice has been modified through recent biochemical research (With, 1947, 1949, Edlund, 1948) If hyperbilirubinæmia has a certain duration, bilirubin invades the tissues and is bound there, presumably to elastin As it is bound to albumin in the serum, this process cannot be a *simple filtration* or diffusion through a membrane tight to colloids, but, as the lymph from the extremities is known to *contain minute quantities* of albumin, the bilirubin probably enters the tissues together with these traces of protein

Jaundice may be caused by hyperproduction of bilirubin (production jaundice), by decreased excretory capacity of the liver *(retention jaundice) and by diversion of the bile secretion* from the bile ducts to the lymph (lymphogenous jaundice) The lymphogenous variety may be due to minute ruptures in the bile canaliculi (regurgitation jaundice) or to a special secretion mechanism (non regurgitational lymphogenous jaundice) The latter must take place in the initial stage of occlusive jaundice, because bilirubin appears so rapidly and while the pressure in the bile ducts is so low that ruptures of the bile capillaries cannot have taken place The forms of jaundice occurring in the clinic are combinations of these three basic forms Occlusive jaundice is a combination of lymphogenous and retention jaundice, in its beginning, the lymphogenous component is non regurgitational, but later, regurgitation may occur Parenchymatous jaundice is a combination of retention and lymphogenous jaundice of the regurgitation type, due to necrosis of *liver cells opening* the passage from bile capillaries to lymph spaces Also intrahepatic obstruction of *fine bile ducts due to exudation may* play a role A rapidly developing intense jaundice is probably *most often lymphogenous, but severe jaundice* may be caused by retention or hyperproduction alone if it has a week or more *to develop*, hence the rapidly developing jaundice *in viral* hepatitis is probably due to necrosis with regurgitation Hæmolytic jaundice may be due to production jaundice

alone or in combination with retention, but pure production jaundice is probably sufficient explanation in most cases. Non hæmolytic hereditary jaundice may be a non hæmolytic production jaundice in which bilirubin is produced in abnormal amounts by synthesis thanks to an inborn error of metabolism similar to the porphyrias and Watson's idiopathic coproporphyrinuria. If this be true there are two forms of production jaundice, a hæmolytic and a synthetic. Icterus neonatorum is a combination of production and retention jaundice, Mollison has shown that blood destruction in the new born is about twice that in later life and that bromsulfalein clearance in the new born is far less than in adults. But the explanation of the typical rise and fall of icterus neonatorum is the Eck fistula effect of the patent ductus venosus Arantii which Beijers (1923) found to be closed just at the time when the jaundice begins to diminish.

I hope I have mentioned the more important problems and new developments in bile pigment metabolism and have made clear that these themes are much more complicated than formerly believed, that they present many unsolved problems, and that most classical theories concerning them need revision.

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APPENDIX

THE BIOLOGICAL SIGNIFICANCE OF THE DIRECT DIAZO REACTION*

TORBEN K. WITH

HIJMANS VAN DEN BERGH (1918) expressed the opinion that in "dynamic jaundice" the bilirubin of the serum has not passed the liver cells, in contrast to "mechanic jaundice" in which the bilirubin of the serum has passed the liver cells and afterwards returned to the serum again. The term "dynamic jaundice" is old fashioned but covers our groups, production jaundice and retention jaundice. As van den Bergh found that the diazo reaction was of the direct type in "mechanic jaundice"—in which is included hepatitis which at his time was believed to be due to obstruction of the bile passages by mucous plugs—and in bile, but of the indirect type in the serum of patients with "dynamic jaundice," it was easy to conclude that bilirubin did react indirectly before passing the liver cells and directly after having performed this passage. This hypothesis which was advanced rather cautiously by van den Bergh was regarded as an established fact by Lepehne (1920) and McNee (1922) and this view has been accepted by most later writers (cf. Watson, 1946). Only a few authors have questioned this universally accepted theory as for instance Harrison (1937, p. 246), Stein (1941), Cantarow *et al.* (1942), Pavel (1943), With (1944), Cantarow and Trumper (1945, p. 441), Jiménez Diaz *et al.* (1948) and Kühn (1950).

A serious objection to the hypothesis that the direct reaction is only given by bilirubin in serum which has passed the liver cells is that the prompt direct reaction is found in the

*Being Chapter X, Section 6, of "Biology of Bile Pigments," a monograph being prepared by Torben K. With.

serum of patients with severe acute yellow atrophy of the liver (cf Castex *et al*, 1940, Stein, 1941, Pavel, 1943, Watson, 1946), for in such cases the damage to the liver parenchyma must be so severe that hardly any bilirubin can pass the liver cells This goes strongly against the hypothesis and this point has to be emphasized because it has not yet been made sufficiently clear

Further, Schaffner *et al* (1949) have questioned the liver passing hypothesis on the basis of much clinical material They feel that it is in the Kupffer cells rather than in the parenchymal cells that the change from the indirect reaction to the direct takes place

Moreover, several experimental observations on obstructive jaundice point decisively against the hypothesis Thus the bilirubin concentration of the serum rises rapidly in experimental occlusive jaundice in rabbits and dogs, but nevertheless the direct reaction only develops gradually Several investigators have observed this phenomenon Lepehne (1921) found a biphasic reaction in the initial stage of obstructive jaundice in dogs, and Kodahma (1925) found the indirect reaction in dogs during the first five to six hours after the occlusion, and the direct after eight to ten hours Necrosis of the parenchyma giving rise to regurgitation was first observed after forty eight hours Davies and Dodds (1927) found no direct reaction in the serum of rabbits with biliary occlusion during the first twenty hours, biphasic reaction after forty hours, and prompt reaction only after sixty six hours Lepehne (1921) found prompt reaction twenty four hours after the occlusion in three rabbits but only after fifty hours in a fourth, and after seventy two hours in a fifth, and similar observations were reported by Retzlaff (1923) and Griffiths and Kaye (1930) In dogs the initial phase, with indirect reaction, is considerably shorter—only a few hours—than in rabbits, as the direct reaction is established about five hours after the occlusion has taken place (Barron *et al*, 1928) These observations are not in agreement with the assumption that the serum bilirubin acquires its direct

reaction by passing the liver cells, as the initial rise in serum bilirubin after biliary obstruction is due to the bilirubin from the liver lymph—this bilirubin has passed the liver cells—and not to retention of bilirubin in the blood, which is known to develop much later than the lymphogenous jaundice (cf Shafiroff *et al*, 1939)

[illegible]

that they have rarely obtained a definite direct reaction under such conditions And they further mention that in some species of animals the diazo reaction is direct in bile obtained from the gall bladder and indirect in bile obtained from the hepatic ducts, and that in species of animals which do not possess a gall bladder the bile never gives a direct reaction They conclude "This would appear to be sufficient to prove that the mechanisms of the direct and indirect van den Bergh tests are not simple"—and this clear and critical view one can hardly but approve, but unfortunately the same authors have completely changed their minds a few years later, as Bollman and Mann (1932) state "In all our experiments, on complete removal of the liver, it has been noted that the bilirubin that accumulates in the blood and tissues is comparable to that found in man normally, and in cases of hæmolytic icterus, that is the indirect van den Bergh reaction is positive but the direct reaction does not occur" This seems contradictory to their statement cited above, but as Mann, Bollman and Magath (1924) mention that after hepatectomy van den Bergh's test is first indirect and later may become biphasic the former statement must be right and the latter cannot be quite correct Further, it has to be remembered that the serum bilirubin concentrations reached in hepatectomized animals are always low, the highest value obtained—and that was after injection of solutions of hæmoglobin—in hundreds of experiments of Mann *et al* (1924 l c) was 3 2 mg per 100 ml, and, as the bilirubin concentration plays a certain role for the direct diazo reaction,

it is perhaps not so surprising that the direct diazo reaction only becomes biphasic and practically never prompt under these conditions Moreover, one has to remember that even after experimental occlusion of the common bile duct the direct reaction only develops after several hours and de hepatized animals do not live much longer As the bilirubin concentration after ligation of the common bile duct plus the cystic duct rises much more rapidly than after hepatectomy— which also causes profound changes in the general bio chemical surroundings"—it is not difficult to understand without the aid of the liver passing hypothesis that the diazo reaction in dehepatized animals only seldom becomes biphasic and practically never direct

Further, it has been shown directly by Gebhardt (1939) that liver passing is not always followed by a direct reaction He found the indirect reaction in the bile under certain conditions Deenstra (1947) made similar observations, and found moreover, that the bile may show the indirect reaction simultaneously with a prompt direct reaction in the serum and he warns against drawing too close parallels from animal experiments to human pathology

More experimental evidence against the liver passing hypothesis has recently been delivered by Snapp et al (1944 1947) who found that intravenous injection of aqueous solutions of bilirubinate in dogs was followed by a hyper bilirubinæmia with indirect diazo reaction, but if before the injection, the bilirubinate solution was mixed with dog plasma and a certain amount of alkali added—which made the reaction of the mixture direct—a hyperbilirubinæmia of the direct type occurred These experiments show clearly that

passed the liver cells, but it certainly raises grave doubt of the liver passing theory

Finally the experiments of Jimenez Diaz et al (1948) con tribute to our doubt of the validity of this theory They

read the direct and total serum bilirubin with the Malloy and Evelyn technique in 15 dogs in various phases of experimental biliary obstruction, and not only in the blood of the general circulation, but in blood both from the abdominal aorta and from the hepatic, portal, renal mesenteric, splenic, and inferior caval veins Bilirubin with indirect reaction was found to enter the blood stream via the splenic vein and to disappear during the passage of the blood through the kidney and intestine, and no signs were found pointing towards transformation of indirect reaction into direct through passage of the liver In conclusion, the authors express the opinion "that the views that are currently held in these [illegible]

passing hypothesis cannot be maintained The very complex chemical phenomena known as the direct and indirect diazo reactions seem hard to correlate to biological processes, and it is perhaps natural at present to regard them as curiosa without biological and clinical significance

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is clinical jaundice, is when the values are higher than 30 mgm per 100 cc of serum In such a case we are almost surely dealing with a hepatic type of jaundice and not with an obstructive one

When the values for total bilirubin are less than 1 mgm per 100 cc of serum, the values for the prompt direct are still more important When we find a total value below 1, and the prompt direct bilirubin is higher than normal, that is to say with an absolute value over 0 25 mgm per 100 cc of serum we are dealing with a case of liver disease (Dr Watson's values are slightly lower than ours, up to 0 2.) In our experience total bilirubin below 1 with a high prompt direct, practically always mean liver pathology And, still more important, when we have found normal persons with a value slightly over 1 for the total bilirubin their prompt direct has always been normal.

C J WATSON I am pleased that Dr Ducci has obtained further evidence of the importance of the prompt direct reacting fraction of the serum bilirubin We feel that we get a good deal more information if we measure the prompt direct as well as the total bilirubin particularly in cases of mild liver disease or so-called latent jaundice The prompt direct fraction is often two to three times the upper limit of normal when obvious pathological conditions are present but often with little or no significant change in the total bilirubin value In hæmolytic jaundice the proportion of prompt reacting fraction is always quite small and in cases of liver disease with a hæmolytic component there is usually a similar but not so marked a disproportion

H POPPER I would like to present some findings of Drs Schaffner, Steigmann and myself (Schaffner F Popper H and Steigmann F 'The significance of bilirubin partition in hepato-biliary diseases', Am J Med Sci 219 307 1950) to support the point which Dr Watson has just made They concern the relation between direct and prompt reacting bilirubin in 70 cases recovering from jaundice This relation was correlated with the evidence of persisting activity of the hepatic process on the basis of clinical or of laboratory findings When the total bilirubin was elevated and the prompt reacting had already returned to normal 11 per cent showed clinical and 33 per cent laboratory evidence of persisting activity In contrast when the total had returned to normal but the prompt reacting was still elevated 35 per cent showed clinical and 70 per cent laboratory evidence of persistent activity In the instances in which both returned simultaneously to normal 17 per cent showed clinical and 50 per cent laboratory evidence of activity This indicates that in the defervescent stage of jaundice elevation of the prompt reacting bilirubin in the presence of a normal or almost normal total bilirubin level may be a good and practically useful indication of the hepatic process With fully developed jaundice the relation between direct and prompt reacting bilirubin is of little diagnostic value except in the recognition of hæmolytic jaundice I also agree with Drs Watson and Ducci that even if the theoretical basis of the diazo-reaction is at present under challenge it is of great value from a practical and clinical point of view

high labelling indicating the second source stercobilin containing ^{15}N is still present

Direct synthesis of stercobilin *from porphyrin precursors is unlikely* [illegible]

jaundice has the same side chains as protoporphyrin except that the two vinyl groups have been reduced to ethyl groups, and that the *arrangement of the side chains is the same* We believe that porphyrins *are synthesized from a monopyrrolic compound, if so it is difficult to* see how by direct synthesis of bile pigment from the precursor you would get the same arrangement of the side chains

We therefore wanted to find out whether the labelled stercobilin which appears soon after labelled glycine was fed was the same as the stercobilin from the hæmoglobin breakdown It seems to be precisely the same, the chemical analysis and the physical properties are the same We therefore examined the infra red spectrum We first showed that you could recognize transposition of groups in a tetra pyrrolic pigment, for example we found the spectra of coproporphyrin 1 and coproporphyrin 3 were quite different We then examined the stercobilin obtained from a case of hæmolytic anæmia, and from a case of congenital porphyria in which the bile pigment is mainly derived from the first metabolic source The infra red absorption spectra were identical We therefore do not think that stercobilin is synthesized [illegible]

practically no breakdown of the hæmoglobin until the end of the life span of the red cell We feel, therefore, that the first source of bilirubin derives from the degradation of hæmoglobin of red cells before they are mature

H DUCCI I would like to say a few words about the clinical implica [illegible] in agreement with Dr With that the [illegible] ver we have to [illegible] of serum as an [illegible] *When we find* a hyperbilirubinæmia we consider the value for the prompt direct bilirubin measured in one minute, according to the general procedure of Malloy and Evelyn When the percentage of prompt direct *bilirubin* is below 35 per cent we consider the case one of prehepatic jaundice, and we make the diagnosis between the hæmolytic and non hæmolytic types according to the excretion of urobilinogen in the fæces When the percentage of the prompt direct is higher than 35 *we are almost always* dealing with a parenchymatous or obstructive type of jaundice In our experience the real importance of the total bilirubin values when there

is clinical jaundice, is when the values are higher than 30 mgm p 100 cc of serum in such a case we are almost surely dealing with hepatic type of jaundice and not with an obstructive one

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found in most normal urines But the gross bilirubinuria found in jaundice can hardly be explained in this way.

N. H. MARTIN · Some of the bilirubin could be passed as a protein conjugate.

T. K. WITH · Yes, it could.

C. H GRAY Dr Gajdos suggested that in urine there might be a constituent of low molecular weight interfering with the various reactions which he performed. Might that constituent be a bile acid present in blood, and might it be the cause of the direct and indirect Van den Bergh reaction ?

A. GAJDOS . Bile salts don't interfere The addition of bile salts does not change the results On the other hand, the urines studied in our experiments have often been free of bile salts

C. J. WATSON · I would like to say a few words about the question

urobilinogen in either fæces or urine Baumgärtel's explanation for that is that there is a block in the liver under those circumstances, and that the necessary dehydrogenase system of the liver cell is suppressed, so that the reduction is not carried out. That, of course, is purely theoretical, and difficult to reconcile with the occurrence of large amounts of urobilinogen in the urine in a variety of states of liver injury

Sborov, Jay and I have been studying the effect of aureomycin on

practically disappears It decreases into the range that characterizes

a complete biliary obstruction, less than 5 mg per twenty four hours It sometimes becomes so little that it is extremely difficult to demonstrate And with this it disappears from the bile and from the urine This in itself would seem to speak very strongly against the idea that mesobilirubinogen which is one of the two urobilinogens, is formed in the liver as Baumgärtel believes I think it testifies quite strongly that the enterogenous source of urobilinogen is the only source, and that bacterial reductive activity is required to bring about the formation of any urobilinogen

In the few days immediately after the cessation of aureomycin therapy, when urobilinogen is returning to the fæces we find a very interesting substance a dextro-rotatory urobilinogen, in contra distinction to mesobilirubinogen which is optically inactive and to stercobilin which is strongly lævo-rotatory We first encountered this substance some years ago in infected bile samples For a few days after cessation of aureomycin the bacterial flora as they are returning to normal have an altered reducing ability It is not quite clear what happens but they are unable to form much if any stercobilinogen during this period It appears that they don't carry out the reduction completely, but only to this dextro-rotatory form That I think has some practical significance The dextro-rotatory urobilinogen as it occurs in the fæces is not as easily extractable by petroleum ether a solvent which has long been used in the quantitative estimation of mesobilirubinogen and stercobilinogen This gives rise to a discrepancy between the simple Ehrlich aldehyde reaction on an aqueous filtrate of the fæces and the aldehyde reaction as carried out on a petroleum ether extract

B Kark We use Dr Watson's twenty four hour technique for study of fæcal urobilinogen excretion and have on occasion found unexpectedly low levels in the stool when we expected large quantities of urobilinogen to be excreted I wonder if Dr Watson can tell us if dextro-urobilinogen is excreted in quantities in the stools of such patients?

C J Watson There is always a slight discrepancy between the simple Ehrlich reaction on an aqueous filtrate and the result obtained with the petroleum ether extract We had hitherto assumed that it was due to non urobilinogen compounds such as indole and skatole because many pyrroles can give an Ehrlich aldehyde reaction if the conditions are right. Now however we are starting a rather broad investigation to find out whether this discrepancy is always explained even in normal fæces, by the presence of a small amount of dextro-rotatory urobilinogen I shouldn't be at all surprised if this were true

T K With Have you isolated this compound in the pure condition?

C J Watson Yes we have isolated it twice in crystalline form It is rather difficult to isolate because it is not as stable as stercobilin We are not sure yet whether it is a stereoisomer of stercobilin but we think it is because it is dextro-rotatory to about the same degree that stercobilin is lævo-rotatory

PART V

SPLANCHNIC BLOOD FLOW

Chairman C J WATSON

RELATION OF LIVER BLOOD FLOW TO CHANGES IN CARBOHYDRATE METABOLISM

SHEILA SHERLOCK

I SHOULD like to speak about the work we have been doing at the Postgraduate School on the relation of liver blood flow to changes in carbohydrate metabolism This work has been done in collaboration with Dr A G Bearn and Dr B H Billing The technique we have been using for liver blood flow is well known to you, it is that of Bradley and his co workers (1945) A catheter is passed into an hepatic vein and the extraction of bromsulphalein by the liver is measured Most people who have used this technique have only continued for a matter of an hour, we have gone on for periods of two to two and a half hours measuring the overall changes in hepatic (splanchnic) blood flow over that time and comparing the changes in flow with changes in hepatic output of glucose and in hepatic lactic acid uptake

We have used adrenaline and noradrenaline to produce the changes Adrenaline differs from noradrenaline chemically in that it has an extra methyl group and it differs very much in its pharmacological actions Both are probably produced by the normal adrenal gland Noradrenaline is an overall vasoconstrictor and adrenaline is an overall vasodilator Adrenaline increases the cardiac output greatly, and noradrenaline has practically no effect Fig 1 shows the effect of these two substances on splanchnic blood flow This is a composite graph for adrenaline it represents mean values

for observations on nine subjects; for noradrenaline it is obtained from the mean of six observations. The dose of adrenaline is 0.1 γ per kg body weight per minute; the dose

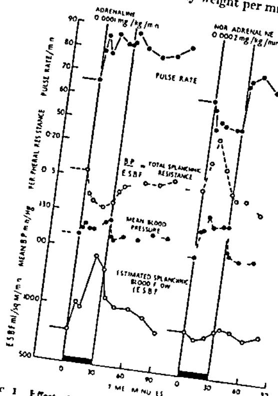


FIG. 1. Effect of adrenaline and noradrenaline on splanchnic blood flow

of noradrenaline is 0.2 γ per kg body weight per minute. The substance is infused intravenously over thirty minutes. Generally the response is as other people have recorded; there is a tachycardia with adrenaline and bradycardia

noradrenaline This latter is probably a vagal reflex The overall effect on the blood pressure is a very great increase in mean blood pressure with noradrenaline, but only slight with adrenaline I would like to remind you that we are measuring splanchnic blood flow, not liver blood flow, because, in spite of all efforts, the portal veins remain sacred and we are actually measuring flow from the arterial supply of the splanchnic area right across to the hepatic vein With adrenaline there is a very great increase in splanchnic blood flow, whereas noradrenaline has practically no effect but, if anything, a depression That means that if we apply a simple formula, splanchnic vascular resistance $= \frac{\text{mean blood pressure}}{\text{splanchnic blood flow}}$ we show that adrenaline is a splanchnic vasodilator, whereas noradrenaline is a splanchnic vasoconstrictor

Where are these changes taking place? There are various alternative suggestions It could be in the splanchnic arterioles, and that seems to us the most likely site Alternatives would be in the sinusoids of the liver, but it has been shown in animals by direct application of adrenaline and direct observation of the liver that adrenaline constricts sinusoids (Seneviratne, 1950) In animals a hepatic sluice gate has been postulated with adrenaline, that is a venoconstrictor mechanism at the exit of the hepatic vein from the liver There is no histological evidence of such a mechanism in man We are all interested now in shunts through the liver and it may be that adrenaline opens up some sort of shunt through the centre of the liver That again is just pure theory Our findings are in contradistinction to the work of Grayson and Swann (1950) on the effect of adrenaline on the splanchnic circulation They have shown that it produces a diminished splanchnic blood flow They used much the same amount as we did, but a quite different technique

Fig 2 shows changes in blood glucose We measure the hepatic venous glucose concentration, and the capillary glucose concentration, which is equivalent to that in an

artery. If the difference between them is multiplied by the blood flow, then we get the total amount of glucose that is leaving the liver. With adrenaline there is a great amount of glucose leaving the liver, whereas with noradrenaline the

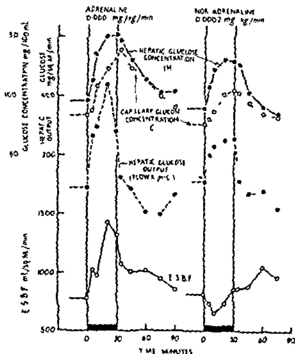


FIG 2 Effect of adrenaline and noradrenaline on blood glucose

glucose output is much less. It is interesting to note that the change in concentration of hepatic venous glucose for these two substances is very nearly the same but the splanchnic blood flow with noradrenaline remains the same or decreases slightly, whereas adrenaline increases the flow and the

resultant output of glucose with adrenalıne ıs therefore much more That ıs reflected very nicely ın the rıse of capıllary blood sugar, that for noradrenalıne beıng about half that for adrenalıne, although the dose ıs doubled There has been

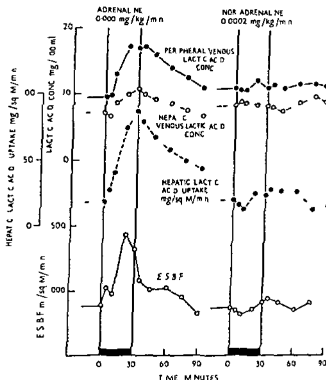


FIG 3 Effect of adrenalıne and noradrenalıne on blood lactic acid

very lıttle work done on the effect of noradrenalıne on carbo hydrate metabolısm, but ın general noradrenalıne ıs belıeved to produce much less effect than adrenalıne

Adrenalıne produces a rıse ın perıpheral venous lactıc acıd (Fıg 3), hepatıc venous lactıc acıd ıs always less than the perıpheral venous value In other words the lıver

removes the lactic acid as it is produced from the muscles. Noradrenaline, rather to our surprise, has no effect on lactic acid metabolism; neither the peripheral venous lactic acid nor the hepatic venous lactic acid concentrations showing any change. We do not know the explanation for this. The lactic acid response to adrenaline is much less than that for glucose.

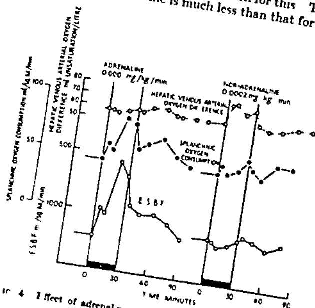


FIG. 4. Effect of adrenaline and noradrenaline on splanchnic oxygen consumption.

It may well be that if we gave more noradrenaline we might produce some sort of lactic acid change. The next obvious thing to do would be to do muscle biopsies and determine whether muscle glycogen diminishes with noradrenaline.

Adrenaline produces no change in the unsaturation of the hepatic venous blood but because the flow has gone up so greatly the actual oxygen consumption of the splanchnic area has increased (Fig. 4). This is in keeping with the

overall findings of oxygen consumption with adrenaline It is known that adrenaline does *greatly increase total oxygen* consumption This increased oxygen consumption certainly is not used for intestinal movements, which are depressed by adrenaline It seems that one of the major needs for oxygen is for the build up into glycogen of lactic acid brought back to the liver from the muscles That fits very nicely with our results, for noradrenaline had no effect on lactic acid *metabolism, and it has practically no effect on splanchnic* oxygen consumption

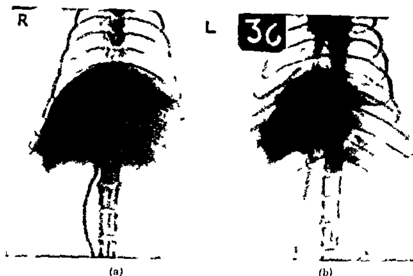
Adrenaline increased hepatic lactic acid uptake and also splanchnic oxygen consumption With noradrenaline, one does notice also that because the flow has been depressed the hepatic venous unsaturation increases to maintain the consumption constant

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(a) (b)

F g 1 In l l l f m

the same phase in its circul ition is seen

(a)

(b) Restricted portal circulation Only the more central and hilar regions of the liver are perfused Compare with (a) and note that the margin of the shadow is hazy and irregular and does not extend to the periphery of the liver Contrast medium is already seen in the thoracic inferior vena cava

*Daniel P M and Prichard M M L (1951) *J Physiol* Vol 112 30 P

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DISCUSSION ANGIOGRAPHIC STUDIES OF PORTAL VENOUS CIRCULATION

M M L PRICHARD

Dr P M Daniel and I have been investigating the portal venous circulation through the liver * Angiographic studies carried out on living animals of various species have shown that the portal venous blood flow may on occasion fail to perfuse areas at the periphery of the liver and may make its trans-hepatic passage through only the more central and hilar regions of the organ When the intrahepatic circulation is thus restricted the blood passes from the portal vein to the inferior vena cava more rapidly than when as has been more commonly the case the liver is perfused throughout

In our study of injected anatomical preparations we have found no communications other than sinusoids connecting the portal and the hepatic venous systems of the liver However the anatomical arrangements of these two systems, together with their connecting sinusoids are in themselves such as to permit in certain circumstances the restricted intrahepatic circulation of portal blood seen in the angiographic studies

This research has indicated that the intrahepatic vessels which transmit the portal venous blood flow are controlled by a neuro vascular mechanism but the exact nature of this mechanism and the stimulus which induces a restriction of the portal circulation such as that observed has not yet been determined The findings are comparable in some respects with those observed in the circulation of the kidney †

Fig 1 shows serial angiograms made during life, illustrating (a) the normal portal circulation (perfusion of all parts of the liver) and (b) the restricted portal circulation (perfu of only the more central and hilar regions of the liver)

I would like to refer once more to the studies presented yesterday *of Elias Petty and myself concerning the distribution of vessels* in human cirrhotic livers as recognized in preparations in which the hepatic and portal veins were injected I want to emphasize again that in cirrhosis many anastomoses between relatively large branches of the portal and hepatic veins can be demonstrated in the connective tissue trabeculæ Such anastomoses must have great influence on the blood flow through the liver in cirrhosis These anastomoses represent many small Eck fistulæ in the liver, possibly through such an anastomosis blood is shunted away from the lobular or nodular parenchyma This should interfere with the blood circulation in the parenchyma In

C J WATSON Was this shown only in cirrhotics ?

H POPPER In the normal human liver such anastomoses cannot be demonstrated To our present knowledge they only develop in the connective tissue trabeculæ of cirrhosis

A GAMMELTOFT Was this after a portal caval fistula had been *performed ?*

H POPPER The studied cases were mostly portal and *a few post* necrotic cirrhosis They were injected after the autopsy In no case had a shunt operation been performed

G R CAMERON You mean that there is anastomosis between the *portal vein and hepatic vein ?*

H POPPER Yes

G R CAMERON It wasn t the hepatic artery ?

H POPPER In the pictures presented the hepatic artery was not injected but only the portal vein with blue gelatin and the hepatic vein with *black india ink In other preparations in which the hepatic* artery was injected in addition communications between branches of the portal vein and hepatic artery were noted

G R CAMERON Methods of determining the blood flow through the liver are unsatisfactory and limited and this one seems to be no

Dr Sherlock with *charac*

have normal variations

moment we are trying

in the liver We have

the

and I said so very feelingly to the members of my team who were mainly responsible for the figures. One of my colleagues confounded me by producing a paper by Dr Blalock, I suppose one of the finest experimentalists in the world, who used similar methods on the dog, and his figures were absolutely identical with those we obtained in the rabbit. This kind of variation seems to be common with all kinds of measurement in the liver.

I think that what Miss Prichard has shown is extremely important. At the moment we haven't any right whatsoever to draw any conclusions about the flow of blood through the liver until we know more about the mechanism concerned. I am quite sure that the methods which we used are almost useless and we'd better start again.

J. Gillman: I had the pleasure of seeing Dr Knisely's living preparations of the circulation of the frog by direct techniques, by transillumination techniques, and by other methods. There is very little doubt in my opinion that the liver cells themselves are subject to a great deal of change. You see a slight swelling of one liver cell coming across a sinusoid. You see the blood diverted in various directions. The effect of food and other physiological factors definitely influence the flow of blood through the liver.

Four or five years ago we showed by injectional techniques, taken for what they are worth, the existence of communications between the hepatic and the portal veins in the normal human liver.

Another point in this connection is the type of reaction which one gets in acute necrosis of the liver. You can get a reaction in which the blood supply disappears and you have almost a coagulative type of necrosis. In different circumstances you get massive pools of blood. I mention this very specifically because the so-called "haemorrhagic necrosis" is a secondary phenomenon and not primary.

J. McMichael: I should like to rise for a moment in defence of physiological methods. Bradley uses the term "estimated hepatic blood flow", and he has also been at pains to point out the great variations which seem to occur. There is no doubt at all that there are very considerable variations in liver blood flow, because you are sampling from one hepatic vein where there may be a fluctuation in flow independently perhaps of changes in other parts of the liver. Even though there are variations, I believe that the method is sound under standard conditions and, if used with proper statistical precautions, the ideas it yields are acceptable. Dr Sherlock's observations on the oxygen content of hepatic vein blood confirmed the general direction of the flow changes which she was obtaining. Reduction of flow was accompanied by greater abstraction of oxygen, which is useful collateral evidence. The methods are the best we've got at the moment, and I think we shall have to go on with them until you give us something a little bit better.

C. H. Best: I don't think this will help to clarify the situation very much. Experimentalists have used animals in which the hepatic artery was completely tied off but as many of you know Dr Markowitz and

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[illegible] the hepatic artery was not [illegible]

[illegible]

by this method you notice that there are variations from [illegible] in normal individuals How can one draw any conclusions about [illegible] of that order ? [illegible] trying [illegible] e have [illegible] of the [illegible]

and I said so very feelingly to the members of my team who were mainly responsible for the figures One of my colleagues confounded me by producing a paper by Dr Blalock I suppose one of the finest experimentalists in the world who used similar methods on the dog and his figures were absolutely identical with those we obtained in the rabbit This kind of variation seems to be common with all kinds of measurement in the liver

I think that what Miss Prichard has shown is extremely important. At the moment we haven't any right whatsoever to draw any conclusions about the flow of blood through the liver until we know more about the mechanism concerned I am quite sure that the methods which we used are almost useless and we'd better start again

J Gillman I had the pleasure of seeing Dr Knisely's living preparations of the circulation of the frog by direct techniques, by transillumination techniques and by other methods There is very little doubt in my opinion that the liver cells themselves are subject to a great deal of change You see a slight swelling of one liver cell coming across a sinusoid you see the blood diverted in various directions The effect of food and other physiological factors definitely influence the flow of blood through the liver

Four or five years ago we showed by injectional techniques taken for what they are worth the existence of communications between the hepatic and the portal veins in the normal human liver

Another point in this connection is the type of reaction which one gets in acute necrosis of the liver You can get a reaction in which the blood supply disappears and you have almost a coagulative type of necrosis In different circumstances you get massive pools of blood I mention this very specifically because the so called hæmorrhagic necrosis is a secondary phenomenon and not primary

J McMichael I should like to rise for a moment in defence of physiological methods Bradley uses the term estimated hepatic blood flow and he has also been at pains to point out the great variations which seem to occur There is no doubt at all that there are very considerable variations in liver blood flow because you are sampling from one hepatic vein where there may be a fluctuation in flow independently perhaps of changes in other parts of the liver Even though there are variations I believe that the method is sound under standard conditions and if used with proper statistical precautions the ideas it yields are acceptable Dr Sherlock's observations on the oxygen content of hepatic vein blood confirmed the general direction of the flow changes which she was obtaining Reduction of flow was accompanied by greater abstraction of oxygen which is useful collateral evidence The methods are the best we've got at the moment and I think we shall have to go on with them until you give us something a little bit better

C H Best I don't think this will help to clarify the situation very much Experimentalists have used animals in which the hepatic artery was completely tied off but as many of you know Dr Markowitz and

they have associations with the names which are familiar and because these diseases have not as yet been characterized fully enough so that they can definitely be called types of hepatitis Our studies have furnished some suggestion that the IH virus like the virus of measles may be antigenically homogeneous The other points of difference indicated on the chart are well known We have seen the A or IH virus with an incubation period of as high as forty days or as low as about fourteen days The work with the SH virus has been carried out with volunteers, and among them we have seen higher incubation periods than this but very rarely lower than 54 days The temperature and symptoms do not help very much to differentiate these two viruses if they can be called two viruses Secondary cases are common with the IH virus and uncommon with the SH virus One important difference and a reason for calling this virus SH is that both viruses appear to occur in blood whereas apparently only the IH virus occurs in fæces We have been unable even with filtrates of fæces from cases of SH injected parenterally to produce the disease in volunteers and cross infection appears to be very uncommon Age susceptibility is also important

In IH epidemics cases are comparatively rare after 40 years of age unless as Dr Bjorneboe has mentioned a population is attacked which may not have had previous experience with the virus Oral administration of IH virus causes infection while orally inoculated SH virus fails to infect With respect to homologous immunity we have repeatedly attempted without success to produce IH viral hepatitis in the same individual twice and such homologous immunity we have also demonstrated in respect to the SH virus Also neutralizing antibodies apparently are produced in convalescent cases

On the other h ... unity between the IH ar ... very clear cut If one ... r the SH virus there was no immunity produced apparently to the heterologous virus This has been demonstrated by injecting

a volunteer first with the IH virus, with production of hepatitis with jaundice, injecting again with the IH virus without production of hepatitis with jaundice, then the SH virus has been inoculated with the production of hepatitis with jaundice a second time, and finally the SH virus has been again inoculated without the production of hepatitis with jaundice We have also proceeded in the opposite direction in volunteers with similar results

The following data on the IH virus (Table II) furnish some suggestion that this virus has a homogeneous antigenic structure This table shows the "pedigree" of the Akiba

Table II

DEVELOPMENT OF AKIBA STRAIN OF IH VIRUS IN CHICK EMBRYO TISSUE CULTURE AND EMBRYONATED HEN'S EGG

Tissue Culture		*Amniotic Series*		*Infection of Volunteers*		
Passage No	*Date*	*Passage No*	*Date*	*Total No*	*No with hepatitis*	*No without hepatitis*
1	8/3/45					
2	17/3/45					
3	24 3/45					
4	30/3/45					
5	24/4/45					
6	13 7/45					
7	18/10/47					
8	30/4/49					
9	7/5/49					
10	24/5/49			6	5	1
	cont d →			4	3	1
		1	8/9 49	4	2	2
		2	19/9/49			
		3	28/9/49			
		↓ cont d		5	4	1

strain of the virus We have also used another similar strain, the New Lisbon strain This work was carried out originally by Dr T N Harris and Dr Gertrude and Werner Henle, starting in 1945 Recently Dr Miles Drake has assisted in this work, particularly in the skin test studies There is a

break in the pedigree because we were unable to procure volunteers However at the sixth passage, in chick embryo tissue culture, grown in Sims Sander's medium, we have been able to pass the virus to volunteers Although there was a clear cut hepatitis, in none of these cases did jaundice occur, whereas generally when volunteers have been inoculated with the natural virus of the Akiba and the New Lisbon strains, jaundice has occurred When four volunteers were inoculated with the eighth tissue culture passage, three developed hepatitis without jaundice, and one remained well And similarly, as shown, volunteers inoculated with the tenth passage material developed hepatitis without jaundice From the tenth passage in tissue culture, the virus was then passed to the amniotic sac of the chick embryo Material from the third amniotic passage produced typical hepatitis without jaundice in four volunteers, while one failed to develop hepatitis The infected amniotic fluid from the third passage n, which represents about 10^{20} dilution from the original fed virus, Akiba strain, was utilized as skin test material t was irradiated with ultra violet light—about five times the amount necessary to kill the PR8 strain of influenza A irus Uninfected amniotic fluid irradiated in a similar ishion was used as control skin test material

For the test there is injected 0 1 ml either of the irradiated nniotic fluid undiluted or diluted 1 2 The test is called ositive if it has induration and redness of 10 mm in diameter, both directions

In those individuals who had the tissue culture passage rus or the amniotic passage virus the days of duration and e clinical signs were similar to those in volunteers who had e natural disease The incubation period was of the usual ngth for the III virus

Cases have not been termed hepatitis without jaundice iless they have had at least three markedly positive liver nction tests on successive occasions in addition to nausea, imiting and a large tender liver

Fig 1 shows the findings in a volunteer with the Akiba

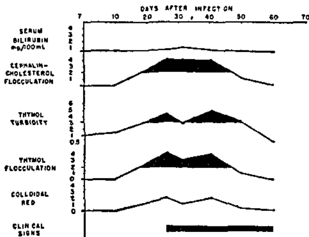


FIG 1 Hepatitis without jaundice following exposure to the 10th tissue culture passage of the Akiba strain (volunteer 23)

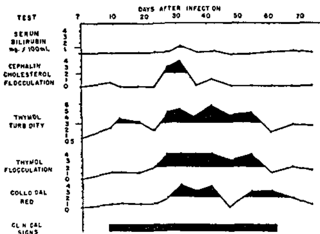


FIG 2 Hepatitis without jaundice following exposure to the 10th tissue culture passage of the NL strain (volunteer 33)

tissue culture passage 10, with typical increases in liver function tests and symptoms of long duration Fig 2 shows another case with the New Lisbon tissue culture passage 10

These volunteers were women prisoners with an exceptional interest in the studies They volunteered more readily because in many cases they were women whom the authorities were able to rehabilitate into civilian life They lost an average of about 10 pounds of weight as a result of the disease caused by the amniotic passage virus and the tissue culture passage virus In addition 80 per cent of them had alterations in their menstrual flow, usually changes in frequency and amount, which we have found in typical cases of infectious hepatitis in young women The performing of bromsulphalein tests on all of the volunteers was difficult because needle punctures for this purpose had to be made twice in the same day It is more difficult to obtain volunteers if one uses the bromsulphalein test as a routine Representative tests, however, indicated a retention of bromsulphalein which was quite characteristic of viral hepatitis Fig 3 shows Akiba amniotic passage 3, also with marked symptoms and increase in the liver function tests

We have carried out approximately 5,000 skin tests The ones that I am going to show are representative These particular ones are shown because they were carried out in certain institutions, and in all of these institutions viral hepatitis was apparently endemic

There were 127 tests in children, of which only 11 were positive In general we have found that in the absence of endemic hepatitis in an institution the children usually have only a small percentage of positive tests Where hepatitis has been endemic, there is a larger number of positive results 286 There are only 8 per cent, however, among the new admissions Among random adults there were 30 per cent, compared with 57 per cent in institutions where hepatitis was endemic Where there has been no endemic hepatitis, only 32 per cent of adults have positive tests The adults differ significantly from the children

The results of the skin test with infectious hepatitis in a large group of individuals were as follows · In the icteric group, of 11 volunteers, 11 were positive (100 per cent); of 136 spontaneous, 128 were positive (94 per cent); averaging 95 per cent for the whole group In the non icteric spontaneous cases resulting from the natural virus, 90 per cent had positive tests, while in the non-icteric induced cases of

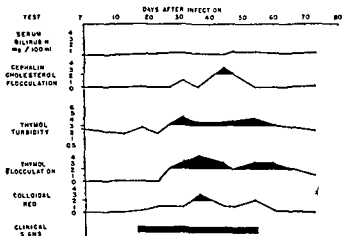


FIG 8 Hepatitis without jaundice following exposure to the 3rd amniotic passage of the Akiba strain (volunteer 28)

hepatitis 97 per cent had positive tests. When the culture virus is used those that have suffered hepatitis without jaundice have positive tests in 100 per cent This and other similar studies would suggest that perhaps the A or IH virus has a common antigenic constitution

In the out patients department of the Children's Hospital, five children were tested during early viral hepatitis, with negative tests They were retested in four to seven days and all had positive tests

Convalescent serum from patients with epidemic hepatitis, added in equal amounts to the infected amniotic fluid, and

incubated, has been used in 3 individuals with known positive skin tests With the neutralized material, their skin tests were negative, with the exception of one patient in whom partial neutralization occurred, the tests before partial neutralization being 25 by 20 mm, and afterwards 10 by 10 mm One other patient had a non specific reaction Serum obtained from patients before their disease developed did not thus neutralize the skin test Six volunteers were negative to skin test during the late inoculation period after exposure to the natural virus Following jaundice in two and hepatitis without jaundice in four, all six individuals developed positive tests In the two cases that developed jaundice the skin tests became positive as the jaundice appeared Five volunteers positive to the skin test, when their resistance was challenged with the natural virus, did not develop hepatitis

In the epidemic in Chicago and at the Rosewood Training School near Baltimore, Dr Drake and I conducted the tests and read them without prior knowledge of the diagnosis In Chicago of 28 new admissions, 25 had negative tests and only three positive tests Among the nurses that had had *hepatitis with jaundice during the period from 1942 to 1949*, 24 out of the 25 nurses had positive tests They also were spotted by the tests without previous knowledge of their history by those performing the tests Thus, in general, correct selection was made purely on the basis of the skin tests

GENERAL DISCUSSION

J H Dible Was the route of infection oral in the infections produced with the attenuated passage strain of virus?

J Stokes Yes

[illegible] these non icteric cases?

[illegible]

instances we have carried out biopsies when there [illegible] without jaundice with the natural virus

[illegible] the test is still

[illegible] e since 1942 [illegible] developed the

J STOKES That is a very difficult problem and we have no answer

SH virus, then finally from infectious mononucleosis, so there are at least those three causes We believe that the individual who has had

N F MACLAGAN I would like to make a short comment on the flocculation tests During the war years we compared around 100 cases of infective hepatitis with about 50 cases of post-arsphenamine jaundice, which we assumed to be mainly the syringe transmitted virus type In that series the infective hepatitis showed about 90 per cent of the flocculation tests and the arsphenamine hepatitis only about 50 per

like to stress again that the work that has been done in America, in Africa by Findlay and his team and in Great Britain has all been done on only one or two strains of these particular viruses That is why I like to use the term viruses rather than diseases I think this work of Dr Stokes and his colleagues has broadened our knowledge of the "singleness" of the epidemic disease In an individual case, the origin

of the virus cannot be identified by clinical, biochemical or histological examination We still do not really know what the virus was in the yellow fever vaccine in the U S A in 1942, other than that it was transmitted by subcutaneous inoculation The same applies in England, except that we were fortunate in being able to recover part of the batch of serum used in our vaccine in 1942, and were able to show that it produced the same high attack rate as the vaccine had We continued to use this plasma for irradiation and other experiments I should like to recall some of our experiments in tissue cultures which were reported in 1944 (MacCallum and Bauer, 1944) * Our source of virus was serum from the cases of yellow fever vaccine jaundice in the American troops in North Ireland in 1942 and convalescent mumps plasma which caused an outbreak in a group of American and English soldiers in Salisbury, looked after by the American Red Cross Harvard Hospital group We passed these two lines of presumed icterogenic material in

Some of these tissue culture supernatants were injected intradermally

reactions and the controls were negative in the convalescents and all were negative in the normal subject, but he developed jaundice 89 days later, I was quite optimistic We then injected the same cultures intradermally in six men who had had yellow fever vaccine jaundice, two who had had plasma jaundice and two who had had ordinary infective hepatitis The skin reactions were all negative in these men so I lost interest in the skin tests However proving that there was infective material there, the normal control in the first experiment

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whether they have done actual transmissions with

*MacCallum F O and Bauer D J *Lancet* 1944 i, 622

DISCUSSION · EPIDEMIOLOGY OF INFECTIOUS HEPATITIS

H DUCCI

The studies to be reported were done in collaboration with Drs. A Horwitz and A Cristoffanini, at Santiago

Although acute hepatitis is so common in Chile, that it constitutes the largest group of hospitalized cases for acute diseases, it is noteworthy that it apparently occurs entirely in endemic form, real epidemic outbreaks being extremely rare

The prevalence of acute hepatitis in our country may be due to the wide use of irrigation in agriculture

We have felt that the absence of epidemics may be due to the constant occurrence of subclinical cases, resulting in immunization of a large proportion of the population In order to test this hypothesis, a study was undertaken of the contacts of a group of 93 consecutive and unselected hospitalized cases of acute hepatitis with clinical jaundice

Due to the lack of specific diagnostic methods, those contacts were considered probably to have subclinical hepatitis, who showed definite positive results in a battery of flocculation tests (thymol turbidity and flocculation, cephalin-cholesterol colloidal gold and colloidal red) The index of positivity was an arbitrary level derived from the results of these same tests in the group of hospitalized cases

These examinations were carried out in a group of 194 known contacts of the hospitalized cases of hepatitis and simultaneously in a group of 223 healthy persons and contacts of patients with other diseases than hepatitis

A study of possible sources of infection was not contributory

The results of this investigation showed statistically significant difference between the hepatitis contact group and the controls, as shown in the accompanying table

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